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A STUDY OF THE LAXATIVE ACTION OF WHEAT BRAN[1]

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Ever since the days of Sylvester Graham,[2] pioneer advocate of the wholesomeness of whole-wheat and Graham flour, the presence of bran in the human dietary has been regarded as more or less beneficial. Wheat bran has long been used as a diabetic food because its carbohydrates are apparently poorly utilized by man. However, in the last few years this supposedly indigestible part of the wheat kernel has taken its place on the breakfast table of millions of Americans along with the cereals of high food value.

This recent popularity of bran in the dietary is due to its reputed excellence as a laxative. In view of the constantly increasing use of bran it seemed desirable to study its physiological effects in some detail and to determine, if possible, what constituent or constituents of bran or combinations thereof are responsible for its well-known laxative action.

In 1906 Jordan, Patten and Hart (1) observed that cattle became constipated when the unwashed whole bran in the ration was replaced by washed bran, and that the constipation disappeared when the washed bran was discontinued and the use of the unwashed bran resumed. These investigators attributed the constipated condition of the cattle to the absence of phytin from the washed bran, and the relief afforded by the un-

[1] The experimental data embodied in this paper are taken from the dissertation presented by George A. Williams in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Yale University, 1926.

A preliminary summary of this work appeared in the Proc. Soc. Exper. Biol. and Med., 1926, xxiii, 630.

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[2] Sylvester Graham (1794–1851), physician, vegetarian and writer on dietetics.

washed bran to the phytin present in the latter. Phytin is very soluble in water and hence was removed during the process of washing the bran.

In an attempt to determine whether phytin has any laxative power, Mendel and Underhill (2) fed the sodium salt of inosite hexaphosphoric acid (phytic acid) to dogs. In some cases there was evidence of laxation, e.g., diarrheal stools, but in others the results were negative.

The prevailing idea in more recent years has been that bran owes its peculiar laxative power to the crude fiber which it contains. However, Smith[3] in 1921 fed dogs bran of various crude fiber contents and observed as great a laxative effect from bran containing a low (4 per cent) proportion of crude fiber as he did from bran of high (12 per cent) crude fiber content. But since the weight of the bran fed in these experiments amounted to 50 per cent of the total food intake, the results can hardly be interpreted in the light of modern bran therapy which employs much smaller quantities of the substance. Holmes (3) after experiments on young men who ingested an average of 144 grams of bran daily in the form of bran bread reported that the subjects showed a variation from "condition entirely normal" to "bowels very loose." This quantity of bran, too, is much larger than that which would be taken by the average individual under normal conditions.

GENERAL EXPERIMENTAL PROCEDURE. Adult dogs ranging in weight from 6.6 to 10 kilos served as subjects of the experiments. The general plan of the tests was as follows: The amount of the standard or control diet necessary to keep each animal in nitrogen equilibrium was determined. The dogs were then given this control diet for a period of eight days. This control period was followed by another eight-day period during which the control diet was supplemented with a measured quantity of bran, or some constituent of bran, or substances similar to bran constituents, or products isolated from bran. The percentage of bran or other supplementary substance which was added to the control food was always based on the weight of the daily control ration.

After each "bran" or other experimental period the dogs were allowed to resume their condition of semi-constipation (characteristic of dogs on the control diet) before being subjected to another "bran" period. Sometimes the "bran" periods were interspersed with regular 8-day control periods. In the case of dogs for which adequate controls had already been established sufficient time was allowed to intervene between periods to restore the animals to their usual condition of constipation.

CONTROL DIET. The control diet was a "synthetic" one consisting of casein, sucrose, lard, cod-liver oil and a salt mixture. It was made up according to the method of Cowgill (4) with this exception: Bone-ash, itself a laxative, was omitted for obvious reasons.

[3] Personal communication from Dr. Arthur H. Smith.

Percentage composition of the control diet

Casein	38.7	per cent
Sucrose	35.5	per cent
Lard	21.5	per cent
Cod liver oil	3.1	per cent
Salt mixture	1.2	per cent
Total	100.00	per cent

The salt mixture used was that of Karr (5) and had the following composition:

Sodium chloride	10 parts
Calcium lactate	4 parts
Magnesium citrate	4 parts
Ferric citrate	1 part
Potassium iodide	0.2 part

Each dog also received about 0.6 gram of "Vitavose"[4] daily per kilogram of its body weight. Cowgill[5] has shown that this amount suffices for the vitamin B requirement of dogs. According to Hoffman (6) "Vitavose" has the following composition:

		per cent
Total soluble carbohydrates:		76.09
Maltose	47.70	
Dextrin	21.10	
Other carbohydrates (by difference)	7.29	
Protein (N × 6.25)		15.36
Ash		5.05
Moisture		3.50
Total		100.00

The cod-liver oil in the daily ration served as the source of vitamins A and D.

The experience of workers in the Yale laboratory (4) has been that dogs living on the above experimental diet do not require vitamin C, and the writer's own experience has confirmed these observations. Dogs fed on this diet for periods ranging from two months to more than a year showed no evidence of malnutrition.

The dogs were fed once daily. The vitavose, bran or other supplementary substance was weighed separately and then thoroughly mixed with the control ration. The animals ate the food greedily during the entire experimental period.

Collection of feces. Feces were marked off with powdered carmine.

[4] A commercial wheat germ extract produced by Squibb.

[5] Personal communication from Dr. George R. Cowgill.

The total weight of dried feces was determined for each 8-day period, and the number of defecations during the same period also observed. Feces were collected fresh whenever possible, and the moisture content determined. The feces were covered with 95 per cent alcohol, and then dried to constant weight on the steam bath.

Criteria of laxation. The number of defecations and the total weight of dried feces per period of eight days were used as criteria of laxation. It was found that both of these quantities were remarkably constant in the control periods of all the animals used, and that the addition of bran and certain of its constituents had a decided effect on both of them. Therefore, by noticing the change in these values it was possible to measure the laxative effect of the substances fed.

Preparation of the bran. The washed bran used in these experiments was ordinary commercial wheat bran which had been washed with water until it was practically starch-free. Bran purchased at a local feed store was tied in a cheese-cloth bag under a faucet and washed with cold water until it gave negative tests with iodine. The washed bran was placed in shallow pans and dried in a current of air at 50°C. The dry product was then ground until it would pass through a 20-mesh sieve, and preserved in a large glass-stoppered bottle containing an open tube of chloroform. The chloroform served as a preventive of vermin.

Composition of washed bran. This air-dried, washed bran had the following composition:

Protein (N × 6.25)	12.50 per cent
Crude fiber	17.24 per cent
Ash	3.12 per cent
Moisture	9.23 per cent
Fat (ether extract)	5.41 per cent
Pentosans	36.94 per cent
Nitrogen-free extract[6] (by difference) other than pentosans	15.56 per cent
Total	100.00 per cent

Analytical methods. The analytical work was done according to the methods of the Association of Official Agricultural Chemists.

Effect of feeding 10 per cent washed bran. In order to observe the laxative effect of bran three dogs A, B and C, were given the control diet supplemented with 10 per cent of washed bran. This bran period of eight days was preceded and followed by a control period of the same duration without bran. The amount of bran given, 10 per cent, was chosen arbitrarily.

[6] In this nitrogen-free extract was included the small quantity of sugar, dextrins, and starch which had escaped removal in the process of washing, and also other complex carbohydrates besides pentosans.

The daily food intake during the control period was 143.4 grams, 114.1 grams and 90.0 grams for dogs A, B and C, respectively. During the bran period dog A received 14.3 grams, dog B, 11.3 grams, and dog C, 9.0 grams of bran daily in addition to the basal diet.

In each case the bran increased the frequency of defecation from 3 to 11 per 8-day period.

The bran also produced an increase in the weight of the feces. But the weight of the feces formed by the bran was always less than the weight of the bran itself. Therefore it seems likely that the increase in feces due to the bran was caused entirely by the undigested residue of the bran. While the bran caused a pronounced increase in the rate of defecation, it did not appear to be conspicuously "feces-forming" in the sense that it caused an increase in the fecal output beyond that which resulted mechanically from its own indigestibility.

It is also probable that the dogs utilized a certain proportion of the bran. At least, all of the bran fed did not appear in the feces.

The feces of the control period were firm and well formed. The bran feces were characterized by the presence of large quantities of bran which apparently had been very little changed in passing through the alimentary tract. While fairly well formed, the bran feces fell apart readily on being touched. This easy disintegration was due to the large amount of bran present. The feces of the bran period were much more moist than the control feces, but there was no evidence whatever of diarrhea. This increase in the moisture content caused by the bran amounted to about five times the control moisture for dogs A and C and about four times the control moisture for dog B. About 2 grams of additional water were excreted in the feces for every gram of bran eaten.[7] The figures for each of the dogs were practically constant, viz.: 2.10 grams for dog A, 1.93 grams for dog B, and 2.02 grams for dog C. This increase in water excretion can be explained in two ways. Either the bran swept the feces through the colon before normal absorption of water could occur or else caused the passage of water from the body into the colon, i.e., in the "wrong" direction.

Minimum amount of bran required for laxation. Smaller quantities of washed bran ranging in amount from 7 per cent to 0.5 per cent, were given to five dogs in order to ascertain the smallest amount necessary to produce any laxative effect. The minimum effective dose of washed bran was found to be in the neighborhood of 2 per cent of the weight of the food eaten. The actual minimum was between 2 per cent and 1 per cent although positive results (an increase in the defecation rate from 3 to 5)

[7] The dogs were allowed water ad libitum in all these experiments, and while the possibility exists that the animals ingested more water while on the bran diet, yet there is no reason to believe that the bran contained any thirst-producing substance.

were obtained in one instance, dog A, with only 0.5 per cent of bran. In this case the weight of bran ingested each day was 0.74 gram or only 82 mgm. of bran per kilo body weight.

In every instance save one, dog D, the increase in the feces was less than weight of the bran fed. In dog D 20 grams of bran produced 37.6 grams of feces, or 17.6 grams more than the weight of the bran. This was the only case in which washed bran produced more than its own weight of feces. In order to confirm this unusual result, this experiment was repeated on dog D, this time with the observation that the increase in the feces amounted to 26.5 grams or 6.5 grams more than the bran fed. So it seemed that in this dog the bran caused the formation of feces over and above its own weight. But this was the only dog in which the bran had this feces forming power. In other experiments also, no matter in what amount the bran was given, in the majority of cases all that the bran added to the feces was a fraction of its own weight, that is, for every gram of bran fed there was formed usually less than one gram of feces. These observations agreed with similar results with 10 per cent bran.

Laxative effect of cellulose (crude fiber). Pure cellulose equivalent to the crude fiber content of 10 per cent bran was given to the dogs, and observations made similar to those of the bran period. Paper pulp[8] was used as the source of cellulose. On analysis it was found that this paper pulp yielded 80 per cent crude fiber. Accordingly crude fiber was prepared by boiling 10-gram quantities of the paper pulp with 1.25 per cent sulphuric acid for thirty minutes, washing the residue free of acid, boiling it with 1.25 per cent sodium hydroxide solution for thirty minutes, washing the resulting material with hot water until it was neutral, and finally drying to constant weight at 110°C. This substance contained practically no ash.

The crude fiber was added to the control diet in an amount equivalent to the fiber content (17.2 per cent) of the bran ingested during the 10 per cent bran period.

It was found that these small amounts of crude fiber which in no case exceeded 2.47 grams daily (dog A) and which in dogs B and C were less than 2.0 grams daily (1.96 and 1.55 grams, respectively) increased the frequency of defecation from 3 to 5 in the case of dog A, and from 3 to 6 in the case of dogs B and C.

The crude fiber also showed some feces-forming properties in two cases, viz., in dog A, 1.0 gram of feces was formed for every gram of fiber and in dog C, 1.5 grams. In dog B the increase in feces produced by 1.0 gram of fiber was only 0.6 gram. But in dog C, 19.0 grams of feces resulted from the ingestion of only 12.4 grams of fiber. Thus it seemed that there was a tendency on the part of the fiber to be feces-forming. It will be

[8] A product of the Penobscot Chemical Fiber Co., Boston, Mass.

recalled (p. 6) that, with one exception, dog D, washed bran showed no feces-forming power, i.e., one gram of bran produced *less* than a gram of feces.

Laxative effect of crude fiber from bran in doses equal to the fiber present in 10 per cent bran. The crude fiber was isolated from bran and given to the dogs in the same proportion in which it occurred in the washed bran. In this way it was possible to test the laxative power of crude fiber obtained from bran and to discover whether this particular constituent of bran was responsible for its laxative effect.

Preparation of crude fiber from bran. The constituents of bran other than fiber were removed by the methods in general use for fiber determinations. Three hundred grams of commercial bran were shaken with 1500 cc. of ether and allowed to stand under the ether over night. This procedure removed most of the fat. The ether was then decanted and the

TABLE 1

Laxative effect of bran crude fiber given in amount equivalent to the crude fiber content of 10 per cent bran

DOG	NUMBER OF DEFECATIONS		INCREASE IN NUMBER OF DEFECATIONS	TOTAL FECES —5 DAYS		INCREASE IN FECES 5 DAYS	WEIGHT OF FIBER FED		FECES PER GM. OF BRAN FIBER	FECES PER GM. OF PAPER PULP FIBER	FECES PER GM. BRAN (10%)
	Control	Bran crude fiber		Control	Bran crude fiber		8 days	Daily			
				grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	
A—8.7 kilos....	3	8	5	39.1	62.5	23.4	19.8	2.47	1.2	1.0	0.88
B—7.7 kilos....	3	5	2	29.8	49.2	19.4	15.7	1.96	1.2	0.6	0.71
C—6.8 kilos....	3	5	2	18.3	38.2	19.9	12.4	1.53	1.6	1.5	0.93

bran, when thoroughly dry, was boiled for thirty minutes with three liters of 1.25 per cent sulphuric acid solution in a large Erlenmyer flask provided with a reflux condenser. The acid mixture was strained through cheese-cloth and washed with hot water until neutral. The residue was then returned to the flask and boiled for thirty minutes with three liters of 1.25 per cent sodium hydroxide solution. The straining through cheese-cloth and washing with water was repeated, and the residue air dried for 24 hours and then dried to constant weight at 110°C. The final product was essentially crude fiber with a very slight proportion of ash (0.8 per cent).

Since analysis had shown that the washed bran contained 17.24 per cent crude fiber, the dogs were given daily an amount of the "bran" crude fiber equivalent to 17.24 per cent of their daily bran (10 per cent) intake. Thus they received the crude fiber freed from the other constituents of bran, and it was possible to measure the laxative power of the fiber uncomplicated by the presence of other bran components.

The effect of the crude fiber from bran when fed apart from the bran is shown in table 1.

It is evident that *gram for gram* the crude fiber from bran produced more feces than either the paper pulp fiber or the washed bran. Bran itself never produced more than 0.93 (dog C) of its own weight of feces, whereas the fiber from bran in one instance (dog C) formed as much as 1.6 times its own weight of feces. Also while a given weight of bran caused eleven defecations, less than one-fifth of the same amount of fiber from the bran caused almost half as many defecations.

The results indicate that whether judged by feces-forming power or by the ability to increase the frequency of defecation, the crude fiber of bran is a far more potent laxative than bran itself.

Laxative effect of very small quantities of the crude fiber of bran. Since a given quantity of the crude fiber of bran seemed to be greatly superior as a laxative to the same quantity of washed bran, further experiments were carried out in which the relative laxative effect of 3 per cent bran, 3 per cent crude fiber of bran, and the "crude fiber equivalent" of 3 per cent bran was determined. By the "crude fiber equivalent" of 3 per cent bran is meant the weight in grams of bran crude fiber equal to the crude fiber present in 3 per cent bran. Since the bran contained only 17.24 per cent of crude fiber, the experiments with the crude fiber equivalent of 3 per cent bran involved the use of relatively minute quantities of fiber, and afforded the opportunity of studying the feces-forming power of fiber from bran when ingested in such small doses as 0.35 gram to 0.74 gram daily.

By feeding 3 per cent crude fiber it was possible to compare the laxative effect of the fiber with that of an equal dosage (3 per cent) of the washed bran.

These comparative tests were made on ten dogs. The effect on a typical dog, dog C, is given in table 2.

The per cent increase in feces based upon the weight of bran or fiber ingested is also listed. This last figure serves as an index of the feces-forming power of each susbstance. If this value is less than 100, it means that the increase in the feces was less than the weight of bran or fiber eaten; if it is greater than 100 it indicates that the supplementary substance caused an increase in the feces of more than its own weight. Whenever the percentage increase in feces (based on weight of ingested substance) was less than 100, the substance was not feces-forming. When it exceeded 100, the substance was regarded as feces-forming. Thus the feces-forming power of each substance can be expressed in mathematical terms, and by the use of this percentage figure and the figures representing the rate of defecation it is possible to express quantitatively the laxative power of the bran and the crude fiber of bran.

The results shown in the following table are typical of the entire series

of experiments. In the case cited the daily ingestion of fiber during the "crude fiber equivalent" period was only 0.47 gram, but it had a marked laxative effect. It caused a daily fecal increase of 1.5 grams, and raised the rate of defecation from 3 to 5 per 8-day period. The increase in the feces for eight days amounted to 319 per cent of the fiber eaten during the same period. On the other hand, 3 per cent bran (2.7 grams daily) caused the feces to increase by only 89 per cent of the weight of the bran itself. But the same quantity of crude fiber (3 per cent) brought about a fecal increase equal to 151 per cent of its own weight. Thus it is evident that the crude fiber of bran was feces-forming when fed in either large or small doses. Similar results were obtained with the other nine animals. Space does not permit the detailed tabulation of the figures for each animal. However, summaries of the laxative effect of 3 per cent bran, 3 per cent crude fiber, and the crude fiber equivalent of 3 per cent bran are given in tables 3, 4 and 5.

TABLE 2

Relative laxative effect of 3 per cent bran, 3 per cent crude fiber of bran, and the crude fiber equivalent of 3 per cent bran

Dog-C Weight 6.8 kilos. Daily control ration—90 grams

	CONTROL	3 PER CENT BRAN	CRUDE FIBER EQUIVALENT OF 3 PER CENT BRAN	3 PER CENT CRUDE FIBER
Number of defecations (8 days)	3.0	6.0	5.0	6.0
Total dried feces 8 days (grams)	18.3	37.5	30.3	50.9
Total bran or fiber intake 8 days (grams)		21.6	3.76	21.6
Increase in feces 8 days (grams)		19.2	12.0	32.6
Daily feces increase (grams)		2.4	1.5	4.2
Daily intake of bran or fiber (grams)		2.7	0.47	2.7
Daily intake of bran or fiber per kilo body weight (grams)		0.397	0.069	0.397
Per cent increase in feces based on intake of bran or fiber		89.0	319.0	151.0

Table 3 shows the general effect of 3 per cent bran on 12 dogs.

As indicated in the table below, 3 per cent of bran caused a decided increase in the defecation rate. This increase amounted to as much as 3 or 4 in every case. In five of the animals the bran seemed to be feces-forming, i.e., it resulted in the excretion of extra feces greater than its own weight. On the average, however, the increment in the feces was practically equal to the weight of the bran fed, and in seven of the dogs the fecal increase was distinctly less than the bran intake. But when the results of the entire series of bran feeding experiments are considered (including

the experiments with percentages of bran other than 3 per cent), the evidence is even more decidedly against the idea that bran is feces-forming. Altogether 24 experiments were conducted in which various percentages of bran were ingested, and in 18 cases out of the 24, the bran was not feces-forming, i.e., the increase in the feces was *less* than the weight of the bran fed. Therefore, while the results obtained in a number of instances during the "3 per cent" periods and in one "2 per cent" case indicated an opposite trend, in the light of the large number of cases (18) in which the results are negative, we are not justified in attributing feces-forming properties to bran.

TABLE 3

Summary of the laxative effect of 3 per cent bran on 12 dogs

SUBJECT	INCREASE IN NUMBER OF DEFECATIONS PER 8-DAY PERIOD	DAILY INTAKE OF BRAN	DAILY FECAL INCREASE	INCREASE IN FECES BASED ON WEIGHT OF BRAN EATEN	DAILY INTAKE OF BRAN PER KILO BODY WEIGHT	FECES FORMED PER GRAM OF BRAN
		grams	*grams*	*per cent*	*mgm.*	*grams*
Dog A	4	4.3	3.6	83	490	0.8
Dog B	3	3.4	2.5	73	446	0.7
Dog C	3	2.7	2.4	89	397	0.8
Dog E	4	3.0	3.5	115	416	1.1
Dog F	3	2.7	4.6	170	385	1.7
Dog G	3	3.0	2.4	78	405	0.7
Dog H	4	2.7	3.8	142	409	1.4
Dog I	4	3.0	3.3	113	428	1.1
Dog K	4	3.0	4.2	141	394	1.4
Dog M	3	3.0	2.1	71	375	0.7
Dog N	3	3.0	2.8	91	400	0.9
Dog O	3	7.5	5.0	67	375	0.6

Also in every case (except one) in which the bran seemed to be feces-forming, the same amount of crude fiber exhibited this feces-forming power to a much greater degree. This greater feces-forming power of the fiber can be partly explained by the fact that the crude fiber was entirely indigestible and hence left more residue in the feces than did an equal quantity of the more digestible bran.

As was seen in table 2, the amount of crude fiber present in 3 per cent bran ("the crude fiber equivalent") was decidedly feces-forming when ingested alone, i.e., separated from the other constituents of bran. Just why 3 per cent of bran, which contained this same amount of fiber, did not show a like power in the majority of cases is problematical. Probably the other constituents of the bran exerted a "retarding" action on the fiber. At least, in experiments to be described later in which a mixture of "bran,"

crude fiber and pentosans was fed to dogs, the weight of extra feces formed did not equal the combined weight of the fiber and pentosans.

Table 4 shows the laxative effect of 3 per cent crude fiber on 9 dogs. The effect of 2 per cent crude fiber on one dog, dog D, is also shown.

As indicated below, the increase in the number of defecations was never less than 3 per 8-day period. In 4 dogs the increase amounted to 4, and in 4 others to 5, while in the case of dog D, there was an increase of 6 in 10 days.

The average daily fecal increase was 4.5 grams and this increase was caused by an average daily ingestion of only 3.0 grams of fiber. In *every* case the crude fiber was feces-forming, and the feces formed amounted, on the average, to 149 per cent of the fiber intake. The percentage increase

TABLE 4

Summary of the laxative effect of 3 per cent crude fiber of bran

SUBJECT	INCREASE IN NUMBER OF DEFECATIONS PER 8-DAY PERIOD	DAILY INTAKE OF FIBER	DAILY FECAL INCREASE	INCREASE IN FECES BASED ON WEIGHT OF FIBER EATEN	DAILY INTAKE OF FIBER PER KILO BODY WEIGHT	FECES FORMED PER GRAM OF FIBER
		grams	*grams*	*per cent*	*mgm.*	*grams*
Dog A	5	4.3	4.8	108	490	1.1
Dog B	4	3.4	5.6	163	446	1 6
Dog C	3	2.7	4.2	151	397	1.5
Dog E	5	3.0	4.5	150	416	1.5
Dog F	4	2.7	5.0	185	385	1.8
Dog G	4	3.0	4.3	142	405	1.4
Dog H	5	2.7	4.8	164	409	1.6
Dog I	4	3.0	4.4	149	428	1.5
Dog K	5	3.0	4.8	160	394	1.6
Dog D (2 per cent) (10 day period)	6	2.0	2.4	120	222	1.2

in the case of 7 of the dogs did not deviate by more than 14 from this average figure of 149. In other words, the amount of feces formed per gram of fiber was fairly constant.

Table 5 gives a summary of the effect of the crude fiber equivalent of 3 per cent bran.

The results in the following table demonstrate most conclusively the laxative power of the crude fiber from bran. Ingestion of small quantities of fiber ranging from 0.35 gram to 0.74 gram daily and amounting to only slightly more than 0.5 per cent by weight of the daily food intake resulted in an average rise of 2.2 in the frequency of defecation and caused the excretion of additional feces equal on the average in normal animals to 390 per cent of the weight of the fiber eaten. The only instance in which a

small amount of fiber was not feces-forming was the case of dog K, but this animal was pregnant during the experimental period.

The increase in the weight of the feces amounted to more than 300 per cent of the fiber intake in all cases except dogs A and B, and in the latter case the increase was more than 200 per cent.

The effect of minimum doses of fiber was decidedly different from the effect of small doses of bran. The bran never produced more than its own weight of extra feces; the crude fiber always did. The bran was partly digested and utilized and therefore did not form so much feces as the fiber which was not at all digestible.

TABLE 5

Summary of the laxative effect of the crude fiber equivalent of 3 per cent bran

SUBJECT	INCREASE IN NUMBER OF DEFECATIONS PER 8-DAY PERIOD	DAILY INTAKE OF FIBER	DAILY FECAL INCREASE	INCREASE IN FECES BASED ON WEIGHT OF FIBER EATEN	DAILY INTAKE OF FIBER PER KILO BODY WEIGHT	CRUDE FIBER INGESTED BASED ON FOOD INTAKE	FECES FORMED PER GRAM OF FIBER
		gram	*grams*	*per cent*	*mgm.*	*per cent*	*grams*
Dog A.....	2	0.74	1.2	162	85	0.51	1.6
Dog B.....	2	0.60	1.3	227	76	0.52	2.3
Dog C.....	2	0.47	1.5	319	69	0.52	3.2
Dog D*.....	4†	0.35(2%)	2.2	640	39	0.35	6.4
Dog E.....	3	0.52	2.0	385	72	0.52	3.8
Dog F.....	2	0.47	2.5	534	68	0.52	5.3
Dog G.....	1	0.52	2.1	399	70	0.52	4.0
Dog H.....	3	0.47	1.5	309	71	0.52	3.1
Dog I.....	4	0.52	2.80	538	74	0.52	5.4
Dog K.....	1	0.52			68	0.52	

* Dog D received the equivalent of 2 per cent bran.

† Ten-day period.

Evidently the laxative effect of the bran was due, at least in part, to the crude fiber present in the bran.

For the purpose of comparison the effects of 3 per cent bran, 3 per cent crude fiber, and the crude fiber equivalent of 3 per cent bran are summarized in table 6.

DISCUSSION. The crude fiber of bran had a marked laxative effect even when fed in doses averaging about 0.5 gram. This effect consisted in an increase in the frequency of defecation and a decided increase (from 2 to 6 times the weight of the fiber intake) in the fecal output. The crude fiber of bran was decidedly feces-forming. This property was not manifested by the bran itself except in a few instances.

Comparison of the effect of 3 per cent bran and 3 per cent crude fiber

shows that, when given in equal doses, the fiber possessed greater laxative power than the bran.

The results indicate that at least a portion of the laxative effect of bran may be attributed to the crude fiber which it contains.

Because of its greater indigestibility a greater residue from the fiber remained in the alimentary tract than was the case when the more digestible bran was eaten. This residue from the fiber probably stimulated peristalsis to a greater degree than the smaller quantity of "bran" residue and in this way may have produced more defecations and also more feces than the bran.

TABLE 6

Summary of the laxative effect of 3 per cent bran, 3 per cent crude fiber, and the crude fiber equivalent of 3 per cent bran

SUBSTANCE FED	AVERAGE INCREASE IN NUMBER OF DEFECATIONS PER 8-DAY PERIOD	AVERAGE DAILY INTAKE OF BRAN OR FIBER	AVERAGE DAILY FECAL INCREASE	AVERAGE PERCENTAGE INCREASE IN FECES BASED ON WEIGHT OF BRAN, ETC., EATEN	AVERAGE DAILY INTAKE OF BRAN OR FIBER PER KILO BODY WEIGHT	AVERAGE WEIGHT OF FECES FORMED PER GRAM OF BRAN OR FIBER
		grams	*grams*	*per cent*	*mgm.*	*grams*
3 per cent bran...	3.4	3.4	3.4	103	410	1.0
3 per cent crude fiber.....	4.5	3.0	4.5	149	399	1.5
Crude fiber equivalent of 3 per cent bran.....	2.5	0.52	1.9	390	69	3.9

SUMMARY

A method is given for measuring the laxative effect of wheat bran and other substances.

Washed bran (air-dried) when ingested in quantities ranging from 10 per cent to 0.5 per cent by weight of the control ration had a marked laxative effect on dogs weighing from 6.8 to 9.0 kilos.

The minimum effective laxative dose of bran for dogs on the diets selected was about 2 per cent by weight of the food intake, or from 200 to 275 mgm. daily per kilo body weight.

When 10 per cent of washed bran was fed to dogs, 2 grams of additional water were excreted in the feces for every gram of bran eaten.

Cellulose prepared from paper pulp was laxative when given in an amount equivalent to the crude fiber content of washed bran. The crude fiber of bran was even more laxative than the cellulose from paper pulp.

Experiments on ten dogs indicated that the crude fiber of bran was

laxative even when fed in the small quantities corresponding to the crude fiber content of 3 per cent bran. Tests with larger quantities (3 per cent) of crude fiber were also positive. Small amounts of the crude fiber of bran caused a marked increase in the number of defecations per 8-day period. The crude fiber of wheat bran was feces-forming, i.e., it had the power of producing more than its own weight of feces. This increase amounted on the average to more than 3 times the weight of the fiber intake. In general bran did not possess feces-forming power to this extent.

The crude fiber of wheat bran is an important contributory factor to the laxative effect of the bran, and, gram for gram, is a much more powerful laxative than bran itself.

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THE RELATION OF SMOOTH MUSCLE TONE TO BIOLOGICAL OXIDATION-REDUCTION

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In a recent paper Voegtlin and Dyer (1926) described a curious phenomenon, i.e., the sensitization of the isolated rabbit jejunum to cysteine. This sensitization is brought about by exposing the tissue, suspended in oxygenated Tyrode solution, to cysteine and allowing sufficient time for the oxygen to oxidize the cysteine to cystine. Less time is required for the sensitization if cystine is used in place of cysteine. In either case the sensitized tissue responds to a subsequent exposure to cysteine by a sudden and great increase in tone, followed by a decrease below normal. This phenomenon was provisionally explained by assuming that exposure of the tissue to cysteine results in a temporary disturbance or shift in the equilibrium of the organic sulphur system:

$$\begin{matrix} R \cdot SH \\ \\ R \cdot SH \end{matrix} \rightleftharpoons \begin{matrix} R \cdot S \\ | \\ R \cdot S \end{matrix}$$

The work of Heffter, Hopkins and Meyerhof has shown that this system is concerned in certain biological oxidations and work from this laboratory has demonstrated that the glutathione system plays a significant rôle in the chemical defense of the body against such specific poisons as arsenic, cyanide, methylene blue, and certain heavy metals (copper and gold). It is of further interest that Voegtlin, Johnson and Dyer (1926) could prove that the toxic action of HCN is primarily due to a chemical action of this poison on the glutathione system. HCN for many years has been regarded as the classical "poison" of biological oxidation, and Loevenhart (1915) has described striking functional changes of the medullary centers resulting from the injection of small doses of cyanide. These facts and considerations suggested a more extensive investigation to determine whether the tone of smooth muscle is partly controlled by oxidation-reduction processes.

The isolated virgin guinea-pig uterus and the rabbit jejunum were chosen as most suitable types of plain muscle structures in view of the fact that under proper conditions these muscles exhibit a remarkable constancy in

tone, and if not stretched too much by excessive weights, respond to a relatively weak stimulus.

Most of the numerous experiments were carried out on the uterus preparation, which was suspended in the usual manner in 50 cc. of Locke solution of the following composition: NaCl 9 g., $CaCl_2$ (anhydrous) 0.24 g., KCl 0.42 g., $NaHCO_3$ 0.5 g., dextrose 0.5 g., and glass redistilled water 1000 cc. The pH of this solution is 7.2. The temperature of the bath was maintained at 38°C. A light heart lever was used and the counter weight was so adjusted as to maintain constancy of tone with small rhythmic contractions for a considerable time before the addition of the substances.

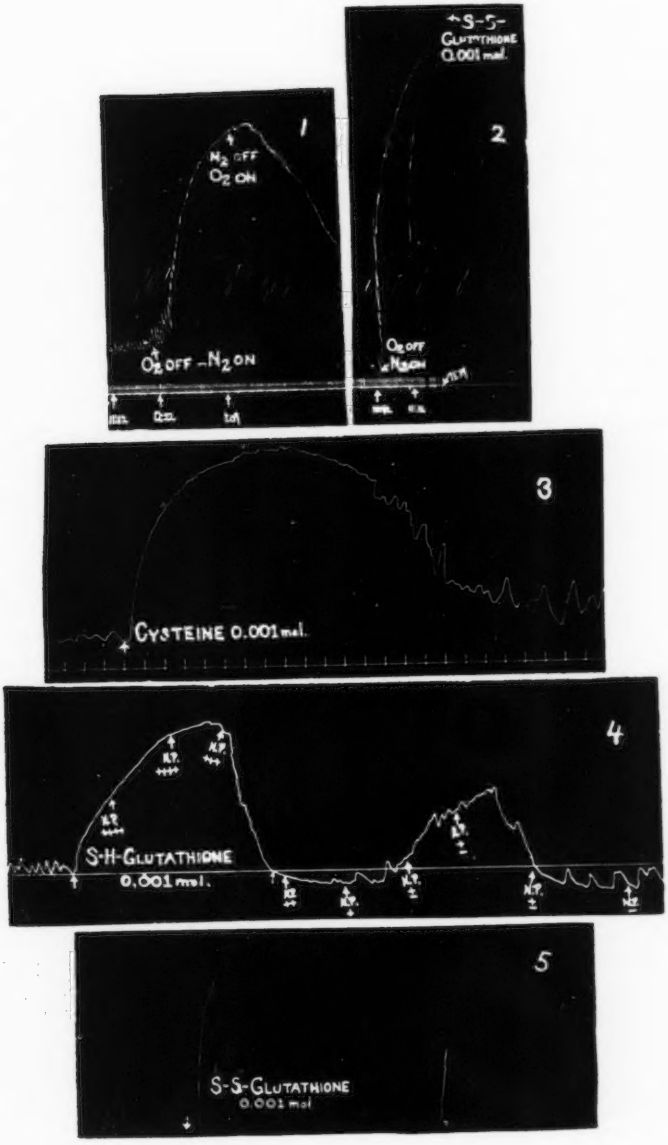
The intestinal segment was similarly treated in Tyrode's solution of the following composition: NaCl 8 g., KCl 0.2 g., $CaCl_2$ (anhydrous) 0.2 g., $MgCl_2$ 0.1 g., Na_2HPO_4 0.05 g., $NaHCO_3$ 1 g., dextrose 1 g., and glass redistilled water 1000 cc. The pH of this solution is 7.4.

As in previous work, the oxygen was passed through a 0.4 per cent $NaHCO_3$ solution in order to supply the gas with a certain amount of CO_2 which seems to have a beneficial effect upon the tissue.

The substances used were chemically pure, prepared by us or obtained from reliable sources.[1] The cysteine was purified by the method of Sakuma (1923) with the exception that glass vessels were used in place of quartz. The substance thus obtained was very pure and did not give the ordinary tests for iron. The glutathione was prepared from yeast according to Hopkins (1921). The substances were accurately weighed on a microbalance, dissolved in a small volume of glass distilled water and adjusted to pH 7.2 by the careful addition of NaOH. The final concentration was then adjusted to 0.1 molar. As soon as the muscle preparations had maintained a constant low tone for about 30 minutes the solutions to be tested were added to the muscle bathing solution by means of an accurately graduated pipette. As the final concentration of the substance in the bath never exceeded 0.001 mol. an appreciable change in osmotic pressure was avoided. The supply of oxygen was abundant and kept constant by means of a pressure gauge. The time on the tracings always represents minutes. By observing these conditions most of the muscle preparations were quite satisfactory for the work and only relatively few had to be rejected. During the experiments the tissue was always exposed to diffuse indirect sunlight as in previous work by Voegtlin, Johnson and Dyer (1924) for the reason that intense sunlight has a marked effect on oxidation-reduction.

As a general rule the reactivity of a guinea-pig uterus which had given no response to a certain substance was checked after washing with fresh

[1] We are indebted to Dr. D. B. Jones of the U. S. Bureau of Chemistry for a sample of valine.


1
N_2 OFF
O_2 ON
O_2 OFF — N_2 ON
2
S-S-
GLUTATHIONE
0.001 mol.
O_2 OFF
N_2 ON
3
CYSTEINE 0.001 mol.
4
S-H-GLUTATHIONE
0.001 mol.
5
S-S-GLUTATHIONE
0.001 mol

Locke's solution by exposing the uterus to a substance which from previous experience was known to produce a marked change in tone.

Experiments with guinea-pig uterus. As previously stated, the general plan was to produce changes in the oxidation-reduction processes of the surviving tissue and to observe the resulting change in tone. It was of interest first of all to study the influence of the withdrawal of molecular oxygen. This was accomplished by replacing the oxygen current in the bath by a current of pure nitrogen. In order to secure complete anaerobiosis, the nitrogen obtained from a steel cylinder was run through a heated tube filled with copper fillings and then through brass tubing to the glass tube of the bath. A layer (1 cm.) of pure liquid petrolatum was placed on top of the Locke solution in order to prevent the introduction of air by the deflection currents produced in the bath by the nitrogen current. Figure 1 illustrates one of these experiments. It will be noted that a change from aerobiosis to anaerobiosis results first in an increase in the size of spontaneous contractions, this being followed by a gradual slow rise in tone until a maximum is reached in about 47 minutes. The tone is kept at a high level for a considerable time (not shown in this tracing). If, now, the tissue is again exposed to oxygen (see fig. 1), the muscle slowly relaxes and eventually returns to a low tonus level. A similar experiment is illustrated by figure 2. In this case there was also a slow rise in tone without loss of rhythmic contractions. After the strip had been exposed for 30 minutes to anaerobic conditions an oxygen-free solution of S−S glutathione was carefully added avoiding the introduction of O_2, with the result that there was an immediate sharp drop to the base line during the following 7 minutes.[2] It is very important to point out that SH glutathione (in place of S−S glutathione) when added under the same conditions does not reduce the tone of the muscle under anaerobic conditions and d-alanine was also found inactive under the same conditions. How can this striking action of S−S glutathione be explained? Previous work by Thompson and Voegtlin (1926) and others has shown that under physiological conditions 90 per cent or more of the total glutathione in normal tissues is present in the reduced (R·SH) form, and Voegtlin, Johnson and Dyer (1926) as a result of a biochemical analysis of the action of cyanide suggested that the glutathione system is essential for the utilization of oxygen by the tissues, the glutathione acting as an oxidation-reduction catalyst. It is therefore reasonable to assume that the complete withdrawal of O_2 would put the glutathione system out of commission by preventing the conversion of the reduced (SH) into the oxidized (S−S) form, i.e., the equilibrium

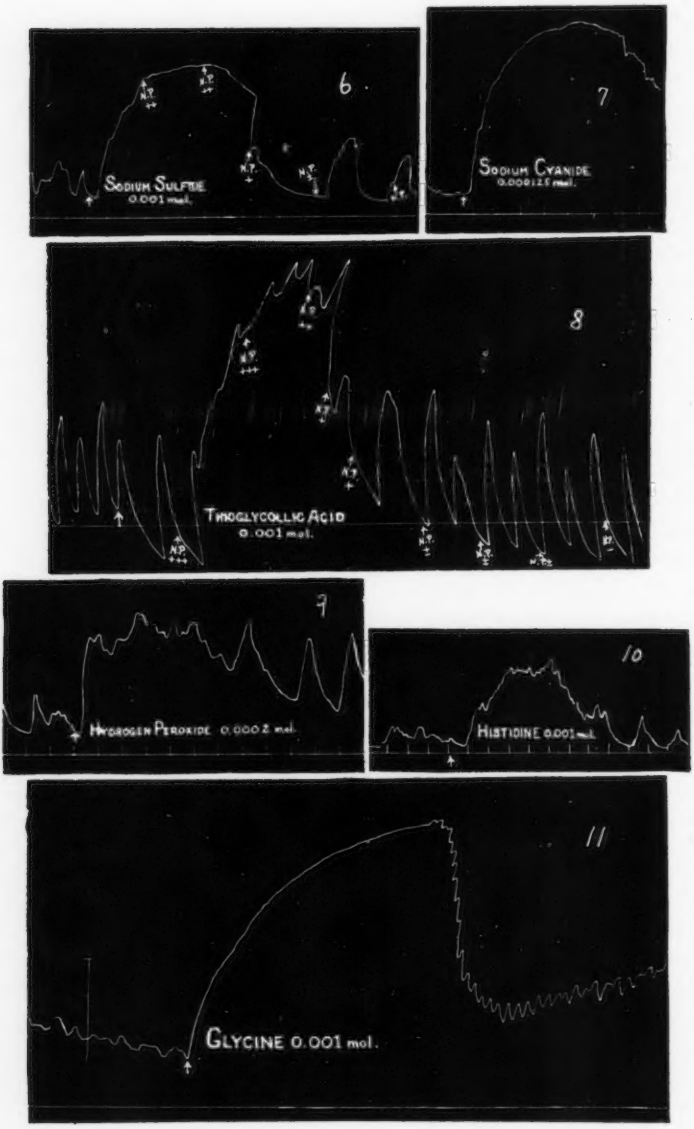
[2] The effect of S−S glutathione could not possibly have been due to the introduction of O_2, as the solution was de-aerated by nitrogen and as the response was ever so much more rapid than when a swift current of O_2 was turned on.

$$\begin{matrix} R \cdot SH & & R \cdot S \\ & \rightleftharpoons & \vert \\ R \cdot SH & & R \cdot S \end{matrix}$$

would be shifted to the left. The effect of anaerobiosis would therefore be the same as if the tissue had been exposed to a sufficient amount of cyanide. As a matter of fact, we shall presently show that the tissue under aerobic conditions responds promptly with a marked increase in tone (fig. 7) upon exposure to a weak cyanide solution. The only difference is that the increase in tone due to anaerobiosis develops much more slowly than that by cyanide. The action of S—S glutathione during anaerobiosis could therefore be rationally explained as being due to a partial restoration of the normal function of the glutathione system. This is an important finding as it demonstrates that glutathione can function in a living tissue in the absence of molecular oxygen.

The next step was to test, under *aerobic conditions*, the action of both forms of glutathione, cysteine and substances which for chemical reasons may be considered as belonging pharmacologically to the same group. The numerous experiments are illustrated by a few representative tracings. Thus figure 3 shows a typical cysteine effect, characterized by a latent period lasting only a few seconds and followed by a rapid increase in tone accompanied by a complete disappearance of rhythmic contractions. In about 8 minutes the contraction has reached its maximum and gradual relaxation takes place with recovery of spontaneous rhythm. Relaxation, however, is not complete and the muscle for some time exhibits an increased tone as compared with that before exposure to cysteine. If the muscle is replaced in fresh Locke solution the tone usually returns to normal. A similar action is also observed with SH glutathione (fig. 4). The effect of both substances is of short duration, indicating some rapid adjustment in the metabolism. It is significant in this connection that after the addition of the mercaptan derivatives the nitroprusside test (N.P.) of the Locke solution gradually becomes weaker and finally negative. At this time analysis of the Locke solution showed that there was still present a considerable portion of the added sulphur, indicating that only part of the substance had entered the tissue, whereas the remainder presumably was converted by oxidation into cystine and S—S glutathione respectively. Figure 5 shows that S—S glutathione also causes an increase in tone. The same effect is therefore produced by a disturbance of the glutathione system in either direction. We considered it unsatisfactory to test cystine, because this substance is practically insoluble at physiological pH and the solutions of its salts show such strong hydrolytic dissociation that a considerable change in pH might be produced by the addition of such solutions to the bathing solution.

Sodium sulphide and sodium thioglycollate in all respects produce the


6
Sodium Sulfite
0.001 mol.
7
Sodium Cyanide
8
Thioglycollic Acid
0.001 mol.
7
Hydrogen Peroxide 0.0002 mol.
10
Histidine 0.001 mol.
11
Glycine 0.001 mol.

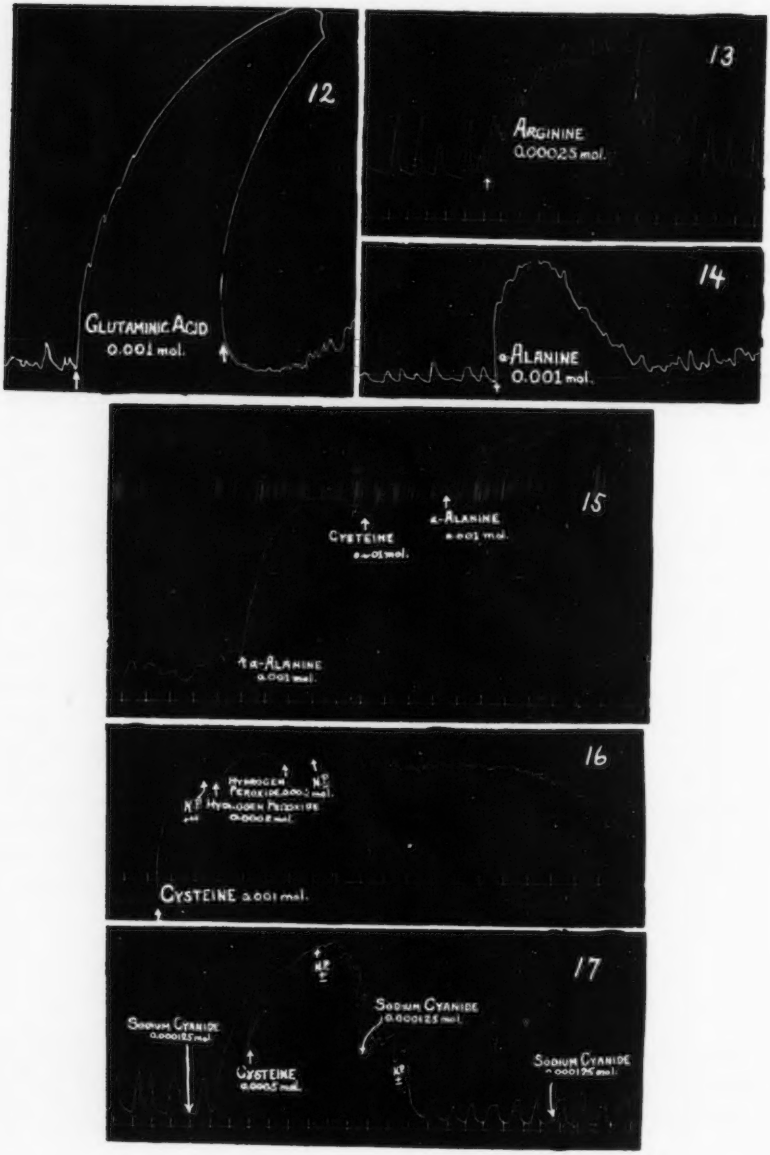
same effect as the other SH compounds (figs. 6 and 8), though thioglycollate was least active and its action could only be demonstrated on exceedingly sensitive strips. Sodium cyanide is the most active substance tested, a 0.000125 molar solution producing a great increase in tone (fig. 7) and slightly higher concentrations produce a tetanic contraction. This is in striking contrast to the inactivity of a 0.001 molar solution of either NaCNO or NaCNS. The temporary nature of the tonus change brought about by threshold concentrations of cyanide might therefore find its explanation in a detoxication of the cyanide by conversion into cyanate (see Voegtlin, Johnson and Dyer (1926)).

Hydrogen peroxide was only slightly less active than NaCN (see fig. 9). A 0.0002 molar solution produced a definite increase in tone and concentrations up to 0.001 mol caused temporary tetanic contractions. From a biological standpoint H_2O_2 is an interesting substance, as its formation as an intermediate product of metabolism has been postulated for a long time, but it is only very recently that its formation by certain anaerobic bacteria could actually be demonstrated. H_2O_2 by its decomposition into H_2O can furnish active oxygen and its effect on the tissue may be explained on this basis.

A further series of experiments dealt with the investigation of the activity of various mono and diamino acids. The information relating to the utilization of these substances by the tissues is still fragmentary and we are unable at present to form a clear picture of the chemical changes to which the amino acids are subjected in the tissues. The synthesis of protein from amino acids is an established fact, but the nature of the transformation of amino acids into non-nitrogenous substances is still poorly understood. Quite recently Knoop (1926) emphasized again the importance of the biological degradation and synthesis of amino acids with particular reference to biological oxidation-reduction. He concludes that the present evidence indicates that the primary degradation of certain α-amino acids involves the following reactions:

$$\begin{array}{l} R \cdot CH \cdot NH_2 \\ \quad | \\ \quad COOH \end{array} \rightleftharpoons \begin{array}{l} R \cdot C = NH \\ \quad | \\ \quad COOH \end{array} + 2H \rightleftharpoons \begin{array}{l} R \cdot CO \\ \quad | \\ \quad COOH \end{array} + NH_3$$

According to this view the catabolism of amino acids would begin with an oxidation, and the energy liberated in the process might very well be used in part for the performance of work. It is highly significant that Knoop found that the reverse reaction, i.e., the synthesis of amino acid from the corresponding keto acid and NH_3 can be accomplished *in vitro* by the use of cysteine as a catalyst. Hence we may conclude that there is some good indication that the oxidation-reduction processes of animal tissues may be affected by exposure to amino acids. The results obtained with the guinea

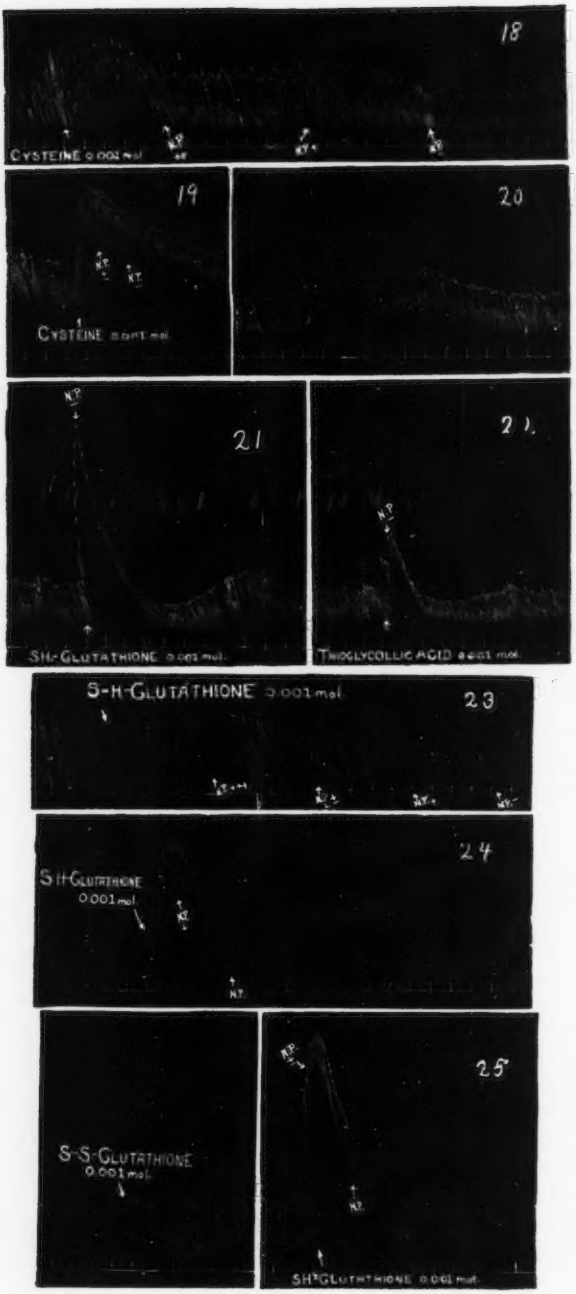

12
Glutaminic Acid
0.001 mol.
13
Arginine
0.00025 mol.
14
α-Alanine
0.001 mol.
15
Cysteine
α-Alanine
β-Alanine
16
Hydrogen Peroxide
Hydrogen Peroxide
Cysteine 0.001 mol.
17
Sodium Cyanide
Sodium Cyanide
Cysteine
Sodium Cyanide

pig uterus clearly show that the following amino acids produce a great increase in tone: α-alanine, arginine, glutaminic acid, glycine, histidine, and valine, whereas leucine, aspartic acid, tryptophane and d,l-α-amino-n-caproic acid were found to be inactive (see figs. 10 to 14). Alanine, glycine and glutaminic acid were most active, the muscle responding almost immediately with a powerful contraction. In the case of histidine and arginine there was a short latent period and the increase in tone was only moderate. Why some amino acids should be active and others inactive in the concentration used (0.001 mol.) cannot be explained, unless it be due to a difference in ability to penetrate into the tissue or differences in utilization.

It was furthermore of interest to determine if some of the substances exhibit a synergistic action. Figures 15 to 17 illustrate some of these experiments. They clearly show that 1, cysteine increases the action of alanine; 2, H_2O_2 that of cysteine, and 3, cysteine that of cyanide. Figure 17 furthermore indicates that in the presence of a small amount of cysteine the increase in tone obtained by a subsequent dose of NaCN rapidly decreases and a second dose of NaCN no longer elicits a response. After the tissue has been washed several times with Locke solution the NaCN again causes the usual increase in tone.

Some experiments were also carried out for the purpose of studying the change of tone caused by SH-glutathione and cysteine in the presence of either atropine, epinephrine or ergotoxin. The presence of these substances in concentrations varying between 1:25,000 to 1:50,000 in no way prevented the action of the sulphur bodies. A curious fact was noted when the muscle was exposed to SH glutathione in the presence of atropine sulphate. In this case the muscle went into a tetanus which persisted for a very long time. Atropine acts therefore as a synergist of SH glutathione.

Experiments with intestinal segment. As previously stated, this work was not as extensive as that with the guinea pig uterus, but a sufficient number of experiments were carried out to show that both from a quantitative and qualitative standpoint the two types of tissue exhibit marked differences. The sulphur bodies: cysteine, SH and S—S glutathione exert only a slight action at the first exposure of the tissue. A small temporary increase in tone is noted. If the spontaneous contractions are somewhat irregular these substances will induce a smooth regular rhythm. Alanine and glycine are inactive. H_2O_2 produces a marked increase in tone. We could confirm previous work (Voegtlin and Dyer, 1926) dealing with the sensitization of the tissue to cysteine by either cysteine or cystine. In a similar way we found that the segment is sensitized to SH-glutathione by either cysteine or SH-glutathione; to thioglycollic acid by either cysteine or SH-glutathione. A preparation which is sensitized to the sulphur bodies does not respond to either alanine or glycine. Alanine, however,


18
CYSTEINE 0.001 mol
19
CYSTEINE 0.001 mol
20
21
SH-GLUTATHIONE 0.001 mol
21.
THIOGLYCOLLIC ACID 0.001 mol
S-H-GLUTATHIONE 0.001 mol
23
24
S-H-GLUTATHIONE
0.001 mol
25
S-S-GLUTATHIONE
0.001 mol
SH-GLUTATHIONE 0.001 mol

will sensitize the segment to cysteine. A typical experiment using cysteine for sensitization is illustrated by figures 18, 19, 20, 21 and 22. Figure 18 illustrates the slight increase in tone and the regulating action on the pendulum movements produced by cysteine. A considerable time is required to oxidize the sulphur in the bathing solution, as is indicated by the persistence of the nitroprusside test. A second exposure to cysteine (without washing of the tissue) made one hour after the first exposure causes a rapid increase in tone (fig. 19), followed by low waves (fig. 20). Subsequent exposures to SH glutathione and thioglycollate give tone increases illustrated by figures 21 and 22. It will be noted that the nitroprusside test rapidly becomes negative. Another experiment is illustrated by figures 23, 24 and 25. This shows that SH-glutathione sensitizes to SH-glutathione, but not to S—S glutathione.

Discussion. Evans (1926) has defined the tonus of smooth muscle as a resistance of its substance to extension. The degree of tonus would be the stretching force divided by the final length of the muscle. Evans regards "contraction as the consequence of certain chemical and physical changes in the cell, and relaxation as their reversal to a former state" and "there should be no difficulty in considering a state of tonus as either a continued contraction, or as an inhibited or partly inhibited relaxation." If we accept this definition the question immediately arises as to the source of energy required to produce an increase in tone. The muscle has to work against a stretching force, in our case the weight of the muscle lever. From a biochemical standpoint this energy might be derived from 1, glycolytic reactions, such as the transformation of glycogen and sugar into lactic acid, or from 2, biological oxidation-reduction processes. Evans (1925) has shown that contracted smooth muscle produces lactic acid, but in smaller amounts than striated muscle. Moreover, Saiki (1908) found that plain muscle destroys added glycogen. On the basis of an extensive study of the cardia sphincter of the terrapin's stomach Snyder (1927) arrives at the conclusion that the contraction of smooth muscle is accompanied by a heat production of about the same magnitude as that of striated muscle. From this and other evidence reviewed by Evans (1926) it appears that the contraction of smooth and striated muscle is associated with the same type of sugar metabolism and it might be reasonable to conclude that at least part of the energy required for the contraction of smooth muscle is furnished by chemical changes in the glycogen-lactic acid system, i.e., from glycolytic reactions. On the other hand our observations definitely show that oxidation-reduction processes also play a rôle. This phase of the subject has hitherto not been studied in a systematic manner and the only information which is available in the literature deals with the relation of O_2 consumption to tonus. Now it should be realized that the determination of the O_2 consumption of living

cells furnishes a relatively simple method for the study of the relation of functional to oxidative changes, but information gained by this means has naturally a limited though important significance. After all, estimations of the amount of O_2 consumed and CO_2 produced by living cells furnish only data on the transformation of O_2 by means of cellular constituents into CO_2 and H_2O. No light is thrown on the *mechanism* involved in this complicated process. In view of the scarcity of information relating to biological oxidation-reduction processes generally it might even appear futile to discuss our own findings, were it not for the fact that they are sufficiently suggestive to permit at least a more or less intuitive treatment.

First of all there can be no question that anaerobiosis of the guinea pig uterus results in a very striking increase in tone and the same effect is produced under aerobic conditions by dilute solutions of NaCN, which according to Evans (1923–1924) cause a large reduction in the O_2 consumption of this tissue. Evans furthermore found that the O_2 consumption of the contracted uterus is smaller than that of the relaxed muscle. He attributes this diminution in O_2 consumption during a period of high tonus to the great reduction of the surface of the tissue. At all events it is obvious that *the energy necessary for contraction cannot be dependent on the supply of molecular oxygen.* But this important fact by no means eliminates the possibility of energy being furnished for this purpose by oxidations which are not primarily dependent on *molecular* oxygen. According to Wurmser (1926) free oxygen intervenes only at the cell surface and the combustions occuring there constitute so-called respiration. In the cell interior there occur complex oxidations and reductions which under *physiological* conditions compensate each other from the energetic standpoint. From what has been said it is obvious that any change produced in this latter oxidation-reduction mechanism may have a profound effect on function. Now we have intimated several times that the organic sulphur system of which glutathione is such an important part, is undoubtedly an important factor in the regulation of cellular oxidation-reduction. How could we explain otherwise the striking action of S—S glutathione on the tonus of the uterus under anaerobic conditions? The addition of this substance evidently restores the balance of R·SH to R·S—S·R. In a similar way we may regard the action of HCN, H_2S, cysteine, and SH-glutathione on the uterus during aerobiosis, as being due to a disturbance in the equilibrium of cellular oxidation-reductions. It is even possible, in the light of Knoop's observations, to attribute the action of certain amino acids to their relation to the glutathione system. And finally it would appear that the sensitization of the intestinal segment also involves a shift in the organic sulphur system.

These considerations are based on biochemical reasoning. It will be of great importance to supplement these data by estimations of the oxidation-reduction potential of the tissue, in order to determine whether the marked changes in tone are perhaps accompanied by similar potential changes. It

is quite conceivable that as a result of anaerobiosis or exposure to SH compounds, HCN, H_2S, etc , the oxidation-reduction potential may drift toward the negative side. A gradual increase in reduction potential during anaerobiosis was observed in the case of bacterial suspensions by Gillespie (1920) and by Cannan, Cohen and Clark (1926) for liver cells and yeast suspensions. These last mentioned workers found that the increase in reduction potential resulting from anaerobiosis is gradual, as is also the increase in tone of the uterus on O_2 withdrawal. This is in striking contrast with the sudden change in tone resulting from exposure to HCN, H_2S and the other substances. Similar sudden potential changes were noted on the addition of methylene blue to anaerobic liver and yeast suspensions.

The tendency for rapid readjustment of tone observed in the aerobic experiments is easily explained by the oxidation of HCN into HCNO, and of the oxidation of part of the SH sulphur into S—S sulphur. The tissue is certainly not injured by exposure to threshold concentrations of these substances as can be easily shown by placing the tissue into fresh salt solution, when it will give the same response to repeated exposures.

In conclusion we do not wish to omit emphasizing again that there evidently exist qualitative and quantitative differences in the reaction of different types of smooth muscle to these substances, a fact which again demonstrates the importance of differentiation for physiological function.

CONCLUSION

Oxidation-reduction processes constitute an important factor in the regulation of the tone of the two types of smooth muscle studied.

Evidence was obtained indicating that the glutathione system is concerned in the regulation of tone during aerobiosis and anaerobiosis.

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THE TOTAL ENERGY REQUIREMENT OF THE ALBINO RAT FOR GROWTH AND ACTIVITY[1]

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In a paper recently published (Hitchcock, 1926) it was reported that the addition of meat to a well-balanced diet, which already contained an adequate allowance of protein, increased the rate of growth of the albino rat to a marked extent. It seemed desirable to determine the cost in calories of this additional growth. From a consideration of the work of Wilson (1902) and Rubner (1908) we should expect to find that the growth of the rats was directly proportional to the available calories in the food consumed. On the other hand, as has been pointed out by Robertson (1923), an animal which is growing rapidly will consume fewer calories per gram increase, other things being equal, than one growing slowly, since in the second case a larger percentage of the total calories consumed is used in the maintenance of the tissue already laid down. This is in accordance with the findings of Aron (1911, 1914).

The experiments reported here fall naturally into three groups. First, the total food consumed by nursing mothers and their litters during the first thirty days of the life of the litters was determined. Second, the total food consumed by young rats from the time of weaning (thirty days old) until they were ninety days old was determined. This, of course, is the period of most rapid growth in the rat. Third and last, the total food consumption of adult rats kept in activity cages was determined. In all cases the food was kept before the rats all of the time, so that the appetite of the rat was the only factor limiting the quantity of food consumed. The nursing mothers and their litters and the rats from thirty to ninety days old were divided into two groups; approximately half of them were designated a control group and were fed a ration consisting of whole wheat flour 62.5 per cent, dry casein 15 per cent, skim milk powder (analac) 15 per cent, $CaCO_3$ 1.5 per cent, NaCl 1.0 per cent, and cotton-seed oil 5 per cent. This food is mixed up in dry cakes which contain approximately 10 per cent moisture. We calculate that one gram of this food contains 3.82 calories, 29 per cent of this total being furnished by protein. The

[1] This investigation has been aided by a grant from the Department of Nutrition of the Institute of American Meat Packers.

remainder of the animals were designated as a meat-fed group and were given once a day all of the fresh meat they would eat in addition to the ration furnished the control animals. In the case of the adult animals kept in activity cages, there was, in addition to a control and a meat-fed group, a low protein group. These animals were fed a ration consisting of whole wheat flour 92.5 per cent, cotton-seed oil 5 per cent, $CaCO_3$ 1.5 per cent and NaCl 1 per cent. This food contained 3.70 calories per gram, 12 per cent of this total being furnished by protein.

TABLE 1

Tabulation of data showing the relative rate of growth and food consumption of meat-fed and control nursing mother rats and their newborn litters for the first thirty days of the life of the litters

	MEAT-FED	CONTROL
Number of litters	14	11
Total number of young	100	81
Total weight of young at birth	617 grams	466 grams
Average weight of young at birth	6.2 grams	5.7 grams
Total weight of young at 30 days	6,178 grams	3,725 grams
Average weight of young at 30 days	61.8 grams	47.3 grams
Total increase in weight	5,562 grams	3,259 grams
Per cent increase in weight of young	901	700
Calories derived from standard ration	44,067	37,657
Calories derived from meat	14,775	none
Total calories	58,842	37,657
Calories consumed per gram increase in weight of young	10.6	11.5
Average weight of mother at birth of litter	221.6 grams	207.4 grams
Average weight of mothers 30 days after birth of litter	221.0 grams	208.6 grams
Estimated caloric requirement for maintenance of mothers	15,687	11,857
Calories available for milk production and for young	43,155	25,800

The quantity of food consumed was determined by weighing the cake of food placed in the food hopper of each cage each morning. The following morning the portion of the food cake that remained uneaten was weighed and the difference in weight taken as the amount of food eaten for that day. In a similar manner the quantity of meat consumed by the meat-fed group was determined. During the early part of the experiment the food was weighed on a Hanson Dietetic Scale, but later a specially constructed Toledo Scale graduated to 0.2 gram was used. Tests made with the Hanson Dietetic Scale showed that the average error with it was not more than 0.5 gram. With the Toledo Scale it was not more than 0.1 gram.

The experiments on nursing mothers and their new-born young were carried out on 25 litters. Fourteen of these containing 100 young were in the meat-fed group. The remaining 11 litters (81 young) were controls, and therefore received no food except the regular stock ration. The data on both food consumption and growth for each group are shown in table 1. It will be seen from this table that the meat-fed group had considerable advantage in the matter of growth. At birth the young in this group averaged 9 per cent heavier than the control young, while at thirty days of age they were 30 per cent heavier than the controls. The 14 mothers in the meat-fed group averaged 6 per cent greater weight than the controls. The meat-fed rats also showed greater economy in the utilization of their food than the controls. Considering the total food consumed by both mother and young, the meat-fed group consumed 10.6 calories for every gram increase in the weight of the young, while the corresponding figure for the control animals is 11.5 calories, in spite of the fact that the meat-fed mothers were heavier than their controls, which of course means a larger number of calories for their maintenance. In a later portion of this paper, it is shown that relatively inactive rats consume about 900 calories of food daily per square meter of body surface. By using this figure it is possible to determine approximately the maintenance requirement of the mother rats in this part of the experiment The figure arrived at is 15,687 calories for the maintenance of the 14 mothers in the meat-fed group during the thirty days that the experiment was in progress and 11,857 calories for the 11 control mothers. Deducting these figures from the total food consumption of the two groups we find that in the meat-fed group 43,155 calories were available for milk production and for the young, while the corresponding figure for the control animals is 25,800 calories.

Jordan (1908) in work on cows has reported that he was able to recover in the milk about 54 per cent of the total available calories of the ration above the maintenance requirement of the animals. If we assume that this figure holds good for rats, then the actual caloric value of the food which the young rats consumed would be 23,304 calories for the meat-fed group and 13,932 for the control group. Now Rubner (1908) has shown that the energy consumed per kilo in doubling the birth weight is nearly the same in all species except man, namely, about 4000 calories. The control animals in this experiment doubled their weight three times during the course of the experiment while the meat-fed animals doubled their weight 3.25 times. Therefore, the number of calories per kilogram of body weight required to double the birth weight of the control animals can be determined by means of the following equation:

$$0.466\,x + 0.932\,x + 1.864\,x = 13{,}932 \text{ Calories}$$

Solving for x we find that 4271 calories per kilogram of body weight were required in doubling the birth weight. Similar computations made for the meat-fed animals give 4189 calories per kilogram of body weight. These figures are in excellent agreement with those given by Rubner for other species. This is the first time this figure has been determined for the albino rat.

At thirty days of age the young rats were weaned and the sexes separated. The experiment was then continued in substantially the same manner for two more thirty-day periods, that is, until the animals were ninety days old. This is, of course, the period of most rapid growth in the life of a rat. One hundred and eleven animals were used in this part of the experiment;

TABLE 2

Tabulation of data showing the average daily food consumption and average daily increase in weight of control and meat-fed rats between the ages of 30 and 90 days

AGE			NUMBER OF ANIMALS	AVERAGE DAILY FOOD CONSUMPTION IN CALORIES	AVERAGE DAILY INCREASE IN WEIGHT	CALORIES PER GRAM INCREASE	PER CENT INCREASE IN WEIGHT
30–60 days	Males	Controls	25	31.46	2.92	10.7	133
		Meat-fed	25	38.09	3.98	9.6	206
	Females	Controls	30	25.72	2.09	12.3	118
		Meat-fed	31	32.02	2.63	12.1	142
60–90 days	Males	Controls	25	41.77	1.68	24.8	32.9
		Meat-fed	25	49.24	2.03	24.2	34.4
	Females	Controls	30	32.87	1.20	27.3	31.2
		Meat-fed	31	38.75	1.13	34.4	25.1

55 of these were controls, 25 being males and 30 females. The remaining 56 animals were in the meat-fed group of which 25 were males and 31 females. These animals were kept in 19 cages, from 3 to 9 animals being kept in each cage. The total food consumption for each cage was determined, in the manner described for the first part of the experiment. The results are shown in table 2. Inspection of this table shows that the caloric cost of a gram increase in weight is inversely proportional to the square root of the percentage increase in weight. Since in all cases except the females from sixty to ninety days of age, the meat-fed rats grew more rapidly than their controls, it follows that their increase in weight was accomplished more economically than that of the controls. In figure 1 the results obtained are shown graphically, the calories of food consumed per gram increase in weight being plotted against percentage increase in weight. The curve obtained is of the general type of rectangular hyper-

bola, the mathematical expression for which is $Y = \frac{K}{\sqrt{X}}$ where Y is the calories consumed per gram increase in weight and X is the percentage increase in weight. The average value of the constant K is 145, and the curve is plotted by the use of this number. However, it is apparent by an inspection of the curve that a majority of the points falling along the vertical portion are to the right of the line while about two-thirds of the points on the horizontal part of the curve fall below. Now the horizontal portion corresponds to the period of growth from thirty to sixty days while the vertical part represents the period from sixty to ninety days of age. This fact, therefore, suggests that the constant is, to some extent,

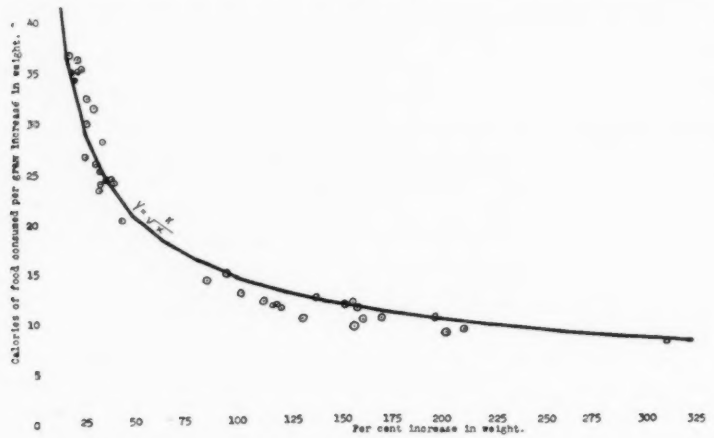

Fig. 1. Curve showing the relationship between the cost in calories of a gram increase in weight of young rats and the percentage increase in weight.

dependent upon age. The data for the period from thirty to sixty days give a constant of 136 with an average variation of less than $\pm$ 6.3 per cent. For the period from sixty to ninety days the constant is 154 with an average variation of $\pm$ 9.1 per cent. If 145 is taken as the constant for both periods, the average variation is $\pm$ 8.9 per cent with 15 out of a total of 38 determinations having a variation of less than $\pm$ 5 per cent.

The final phase of these experiments deals with the relationship between the total voluntary activity of the animals as measured by the revolving cages described by Hoskins (1925) and Durrant (1924, 1925) and the food consumption measured in calories. In all, 62 animals were used for a total of 126 determinations. In each case both activity and food consumption were determined for at least five days and the average of each obtained.

In many cases the determinations lasted as long as twenty days. The animals were arranged in three groups according to the diet which they were receiving. Thirty animals upon which a total of 54 determinations were made were in the control group and received the regular stock ration

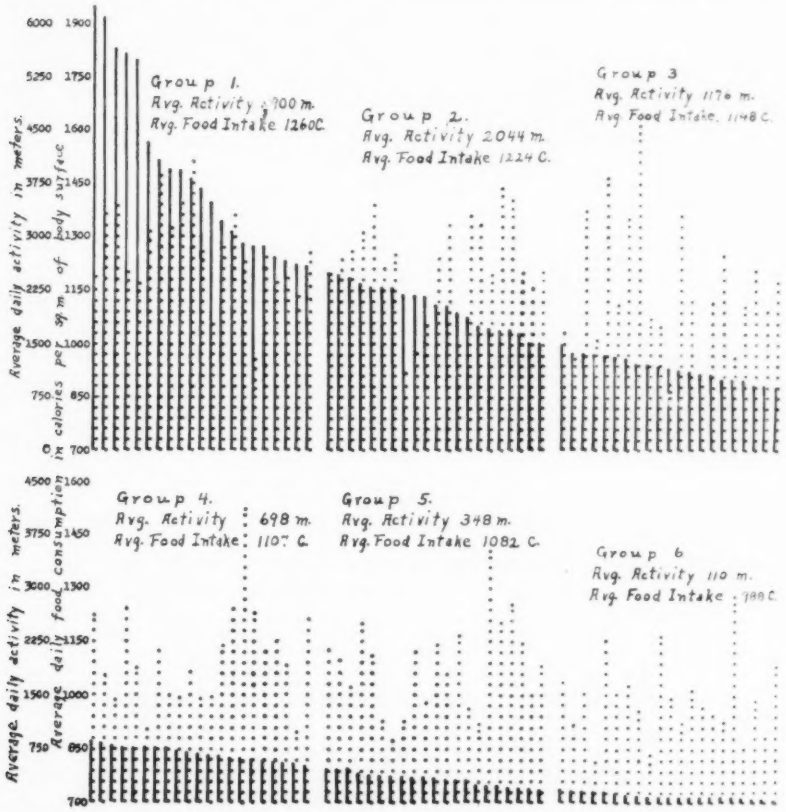

Fig. 2. Graphic representation of the relative bodily activity and food consumption expressed in calories per square meter of body surface in 126 determinations on 62 albino rats. The continuous line represents activity and the dotted line food consumption.

described above. Twenty-four animals upon which 49 determinations were made received meat in addition to the stock ration while 8 animals upon which 23 determinations were made received no food except the low protein ration already described. A careful study of the data fails to show any effect of this variation in diet and the three groups have therefore been

studied together. The results are shown graphically in figure 2. The animals have been arranged in order of decreasing activity, the most active animal being placed at the extreme left. The continuous line represents the activity of the animal while its food consumption expressed in calories per square meter of body surface is shown by the dotted line. The surface area was determined by the use of Meeh's formula taking 11.36 as the constant, which is the value determined by Carman and Mitchell (1926). The figure shows a high degree of individual variation which means that there must be other factors than the animal's activity involved in determining the food consumption. In the light of the recent work that has

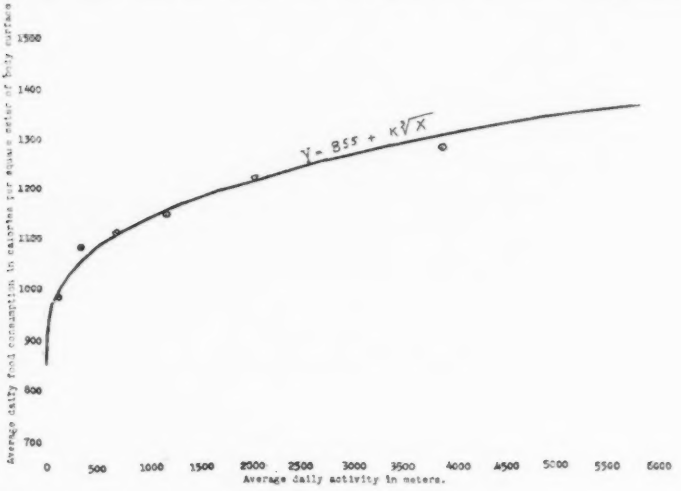

Fig. 3. Curve showing the relation between total bodily activity and food consumption in the albino rat.

been done by Strouse (1924) and his colleagues, this is exactly what we should expect. When individual determinations are plotted, this variation is so great that it is impossible to draw a curve representing the effect of activity upon food consumption. However, if the determinations are averaged in sufficiently large numbers, such a curve can be obtained. For this purpose the results were separated into 6 groups of 21 determinations each, on the basis of activity. The averages obtained from this arrangement have been plotted in figure 3 and a smoothed curve drawn through these points. This curve can be represented by a mathematical expression: $Y = A + K\sqrt[3]{X}$, where Y is the food consumption of the animal expressed in calories per square meter of body surface, A is the caloric require-

ment of a completely inactive rat, and X is the activity expressed in meters. K is, of course, a constant.

The numerical value of A determined by plotting food consumption against the cube root of the activity and projecting the straight line obtained in this way through the line representing zero activity, is 855 calories. Mitchell and Carman (1926) have reported that the basal metabolic rate of albino rats is about 600 calories per square meter of body surface for males and about 570 calories for females. Lee in experiments as yet unpublished has obtained substantially the same figures for rats in our colony. Mitchell and Carman also state that the specific dynamic effect in rats is very slight. The excess above this basal requirement consumed by the animals in these experiments must, therefore, be chiefly due to lack of efficiency in the use of food. The value of K has been determined by dividing the total calories consumed minus 855 by the cube root of the activity expressed in meters. The average for the six points on the curve is 28.58. The average variation is $\pm$ 5.25 per cent. Four of the six points have a variation of less than 3 per cent, while the remaining two points have a variation of approximately 10 and 13 per cent respectively. This seems very good agreement when the high degree of variability shown by the data is taken into consideration.

SUMMARY

1. The relation of food consumption to the rate of growth and total bodily activity of the albino rat has been investigated. On the basis of total food consumed by both mother and young, control rats consumed 11.5 calories per gram increase in weight during the first thirty days of life. Meat-fed rats grew more rapidly and consumed 10.6 calories per gram increase in weight. It has been computed that the control rats consumed 4,271 calories and the meat-fed rats 4,197 calories in doubling their birth weight.

2. During the period from thirty to ninety days of age the caloric cost of a gram increase in weight is shown to be inversely proportional to the square root of the percentage increase in weight.

3. In adult rats kept in activity cages the increase in food consumption due to increased activity is directly proportional to the cube root of the activity expressed in meters. It is computed that a completely inactive rat would consume approximately 855 calories per square meter of body surface. The high degree of variability found in these data leads to the conclusion that there are other factors involved in determining the total food consumption.

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SEX-DIFFERENCES IN HEART ACTION

I. Duration of Systole

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The same methods were employed in determining the duration of the systoles of women as have been described in a previous paper dealing with the systoles of men (1), and the data which we obtained were handled in the same manner. The basis of this paper is a series of 74 tests on 68 women standing, 58 tests on 58 women sitting, and 58 tests on 58 women recumbent. Inasmuch as we read 15 cycles in each test, 2850 cycles were studied.

To be of any practical importance, the length of the systole in every possible cycle length would have to be known, and it would be impossible to determine this by actual tests for all positions studied. We therefore decided to establish what we called standard curves, proceeding as follows, using the same method as we had previously used in the case of men. Employing the data which we had obtained from women standing, we found the average length of the cycles and systoles of all cases studied, the average length of the cycles and systoles of those whose cycle lengths were longer than the average, and of those which were shorter than the average.

Using the three sets of figures thus obtained, together with similar figures from women in the sitting and recumbent positions, and corresponding groups of figures from men,—all of which were reported by us in tables 1 and 2, page 275 in our last paper in This Journal, 1926, lxxvii,—we developed the following formulae, and the curves shown in figure 1.

The formulae allow the calculation of the length of the systole for any cycle length and any of the positions studied. In these formulae S = Systole and C = Cycle.

Formulae

Men, standing at rest	$S = 0.150\ C + 0.1242$
Men, sitting	$S = 0.147\ C + 0.1478$
Men, recumbent	$S = 0.105\ C + 0.2010$
Women, standing at rest	$S = 0.157\ C + 0.1375$
Women, sitting	$S = 0.135\ C + 0.1683$
Women, recumbent	$S = 0.065\ C + 0.2478$

The curves of figure 1 were plotted with cycle lengths as abscissae and systole lengths as ordinates.

Table 1, which corresponds to a similar table for men, recorded in our previous paper, is of some interest as giving a general view of the changes in systole length of women in different positions, and changing cycle and pulse lengths.

I. The curves of figure 1 show several points of interest which apply to both men and women and as we have previously shown, these relations

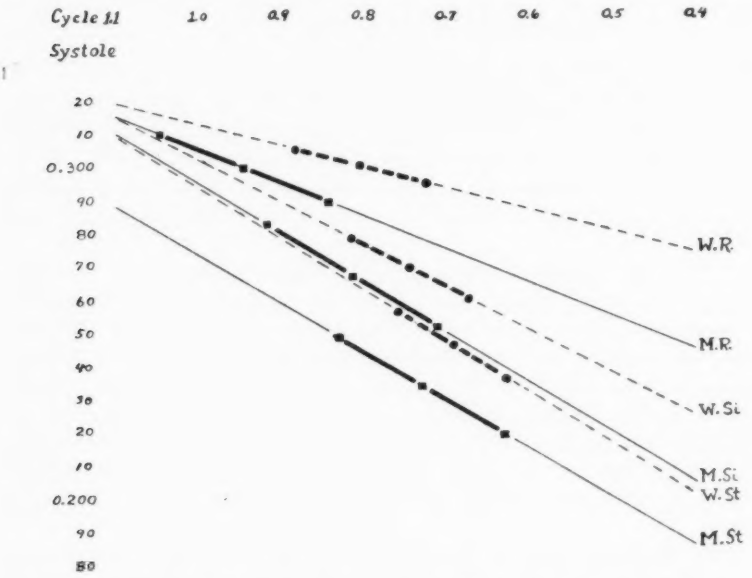

Fig. 1. Curves of the duration of systoles with respect to cycle lengths, cycle lengths being plotted as abscissae and systole lengths as ordinates. *W. R.*, women recumbent; *M. R.*, men recumbent; *W. Si.*, women sitting; *M. Si.*, men sitting; *W. St.*, women standing; *M. St.*, men standing.

can be explained as the result of the interplay of the two factors, cycle length and gravity.

a. The systoles in all positions progressively shorten as cycle lengths decrease. As cycle lengths shorten, (pulse rates increase), not only is there less time for venous blood to accumulate, but the blood is pumped out of the veno-auricular reservoir more rapidly, and the systoles become shorter and shorter as the cycles decrease.

b. The systoles are longer in the recumbent position than in sitting, and in sitting than standing, for like cycle lengths, because gravity retards

the flow of venous blood in increasing proportion as the position approaches the horizontal.

c. The relative pitch of the three curves indicates that as the cycle lengths decrease the systoles shorten less rapidly also as the position becomes more horizontal. In long cycles (slow rates) there is time for the blood to get back to the heart in spite of the retarding effect of gravity in the standing and sitting positions; but as cycles shorten, gravity has a progressively greater influence, and although the effect of short cycles on the accumulation of venous blood is to be seen in all positions, it is more marked in the sitting, and still more in the standing curve.

TABLE 1

Effect of position on the average length of systole at different cycle lengths and pulse rates in the case of women

The duration of the cycles and systoles is stated in seconds

CYCLE LENGTH	PULSE RATE	STANDING	SITTING	RECUMBENT
1.200	50.0	*0.3259	*0.3303	*0.3258
1.100	54.5	*0.3102	*0.3168	0.3193
1.000	60.0	0.2945	0.3033	0.3128
0.900	66.6	0.2788	0.2898	0.3063
0.800	75.0	0.2631	0.2763	0.2998
0.700	85.7	0.2474	0.2628	0.2933
0.600	100.0	0.2317	0.2493	0.2868
0.500	120.0	*0.2160	*0.2358	*0.2803
0.400	150.0	*0.2003	*0.2223	*0.2738
0.300	200.0	*0.1846	*0.2088	*0.2673

The values marked * were calculated on the assumption that the rate of change in the length of the systole would be maintained outside the limits of actual observations.

II. Although the curves of men and women show that the systole lengths respond in general in like manner to the effects of heart rate and of gravity, they respond in different degrees.

a. The systoles of women are longer than those of men for like cycle lengths in all positions. This fact is to be seen by a glance at figure 1. We were uncertain as to the explanation of the fact that the systole of the left ventricle of women is longer than that of men in all positions at the same pulse rates. We first thought that the hearts of women might be less vigorous than those of men, because we had a few cases of women who had exercised considerably, whose systole lengths approached those of men. Through the kindness of Prof. W. P. Bowen, of the Ypsilanti State Normal College, we were able to study this question. He brought to the Physiological Laboratory in Ann Arbor, 19 women students, who had been exercising a great deal for two years, and who were in excellent

condition, as he said, "always on their toes." The length of the systoles of the hearts of these women was tested, and was compared with those of 42 other women students, who were presumably normal and of about the same ages, but most of whom had not given unusual attention to athletics. No differences were found which indicated that physical training had any noticeable effect on the length of the systole.

Bazett (2) says that he was not able to detect any effect of exercise on the duration of the systoles of the women he examined. As a matter of fact, we had not been able to discover, in our work with men, that the systoles of those who were recognized as athletes differed consistently from those of ordinary untrained men.

Apparently the fact that the systoles of women are longer than those of men must be accounted for on the basis of a sex difference.

The fact that the duration of the systole is intimately related to the amount of blood that the heart has to pump suggests that the longer systoles of women may be due to their hearts having more blood to pump relative to their size than those of men. This might be because the return of the venous blood to the heart is more rapid in the case of women than men, or what seems more likely, because the hearts of women are smaller in proportion to the size of body than those of men. Such a sex difference might have arisen through a greater amount of physical activity on the part of the male, even though the amount of exercise taken by different individuals of a given sex has not been found to influence the duration of the systole. Müller (3) found that the weight of the heart of a man is about 10 per cent more than that of a woman of equal body weight. Bardeen (4), who studied the size of the heart by means of the x-ray, found a decided difference between the size of the hearts of women and men.

b. For any given position the pitch of the curves of women differs from that of men.

TABLE 2

	MEN	WOMEN
Standing	0.0150	0.0157
Sitting	0.0147	0.0135
Recumbent	0.0105	0.0065

The pitch of the curves may be expressed by the rate of change in the length of the systole in seconds, per shortening of cycle length of 0.100 second, as shown in table 2. The difference in pitch, as may be seen from this table, is not the same for different positions, and the effect of position on the pitch of the curve is greater in the case of women.

The curve of the systole lengths of women falls less steeply in the re-

cumbent position than does that of men, slightly less steeply in the sitting position, and a little more steeply in the standing posture.

How may these differences be explained? The combined effects of gravity and changing cycle lengths would be greater if the hearts of women were small as compared with men. Decreasing cycle lengths would act like gravity to lessen the amount of blood to be pumped per beat, and like gravity would be more effective in the smaller hearts of women than those of men. In the recumbent posture, because of the large amounts of blood returning to the heart, the shortening of the cycle lengths would have comparatively little effect to shorten the systole lengths of women.

In this paper we have emphasized the importance of the quantity of venous blood supplied to the heart and the way this influence is modified by position of subject. There is another factor which helps to determine the length of the systole, viz., the pulse rate, to which we have referred, but which we have not examined in detail. In the course of our work we became more and more interested in the differences in the pulse rate of women as compared with men in different positions and the effect of it on the length of systoles. We shall follow this paper by another dealing with this subject.

SUMMARY

An examination of the curves of figure 1 shows certain points of likeness between the length of systoles of men and women, viz., *a*, systoles shorten with decreasing cycle lengths; *b*, systole lengths change with change of position; *c*, the pitch of the curves changes with changes of position.

The curves also show points of distinct difference between the sexes, viz., *a*, systoles of women are constantly longer than those of men at the same cycle lengths and the same position; *b*, the rate of fall of the curves of women is different from those of men and this difference varies with the position.

We have emphasized the effects of gravity and of changes in cycle length on the length of systole and suggest that the differences between the sexes may be due to the hearts of women being smaller in comparison with their size than those of men. If such is true, it would explain why the effects of gravity and of changes in cycle length would be greater in the case of women than men.

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SEX-DIFFERENCES IN HEART ACTION

II. Heart Rate

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We wish in this report to refer to the effects of the pulse rate of women as compared with men, as a factor influencing the length of systole in different positions of the body. Incidentally, we shall try to account for the generally accepted fact that the average pulse rate of women is more rapid than that of men.

In our first paper on the subject of the length of the systole of the left ventricle (1), we measured curves obtained from the carotid pulse and estimated the lengths of the cycles and systoles therefrom, calculating the pulse rate from the data thus obtained. We therein pointed out, as others had previously done, that the length of the systole is influenced by the heart rate.

It is advisable to make a distinction between the effect of the cycle length and of the repetition of the cycles which we speak of as the pulse rate. The cycle length is the time in seconds during which the blood can accumulate in the veno-auricular reservoir; the pulse rate is the frequency with which the heart acts, and pumps the blood out of that reservoir. This distinction is to be borne in mind, as we consider the relation of cycle lengths, pulse rates and systole lengths, in the following discussion.

We have several times had occasion to call attention to the fact that the length of the systole of the left ventricle depends very largely on the amount of blood accumulating in the veno-auricular reservoir, and that this is greatly influenced by gravity and heart rate (1). The importance of gravity as a factor in determining the length of systoles of women as compared with those of men in different positions of the body was especially emphasized in our last paper (2).

The manner in which pulse rate influences the systoles of man was also discussed in our first paper (1). The systoles of women are in general influenced by the same factors as those of men, but to different degrees, and the fact that they do not change with changing pulse rates in the same amount certainly needs explanation.

First we wish to call attention to table 1. In preparing this table, we obtained the average of all the cycle lengths in women in a given position,

and the average of those which were respectively shorter and longer than the average of the total. This gave us three groups, which we entered in the table as Longer, Average, and Shorter Cycle lengths, placing the corresponding average pulse rates and systole lengths in the same line. The same was done for men, and for each of the positions studied. The difference in data for men and women was then entered in each case.

If one examines the figures in table 1, he finds:

I. In the case of both women and men,

a. The pulse rate decreases as the position becomes more horizontal.

b. This change in rate becomes more marked the more rapid the rate of the heart.

c. Systoles lengthen as the position approaches the horizontal.

TABLE 1

CYCLE	STANDING			SITTING			RECUMBENT		
	Cycle	Pulse	Systole	Cycle	Pulse	Systole	Cycle	Pulse	Systole
Longer:									
Women	0.7604	78.90	0.2570	0.8160	73.53	0.2785	0.8845	67.83	0.3053
Men	0.8314	72.17	0.2490	0.9196	65.35	0.2831	1.0466	57.33	0.3110
	−0.0710	+6.73	+0.0080	−0.1036	+8.18	−0.0046	−0.1621	+10.50	−0.0057
Average:									
Women	0.6952	86.31	0.2467	0.7477	80.24	0.2693	0.8057	74.47	0.3003
Men	0.7324	81.92	0.2341	0.8156	73.57	0.2677	0.9453	63.47	0.3003
	−0.0372	+4.39	+0.0126	−0.0679	+6.67	+0.0016	−0.1396	+11.00	0.0000
Shorter:									
Women	0.6301	95.22	0.2364	0.6794	88.31	0.2601	0.7268	82.55	0.2952
Men	0.6333	94.74	0.2192	0.7117	84.30	0.2523	0.8441	71.18	0.2897
	−0.0032	+0.48	+0.0172	−0.0323	+4.01	+0.0078	−0.1173	+11.37	+0.0055

d. The greatest change of pulse rate is seen in a comparison of the recumbent with the standing position, when the cycles are short (pulse rates rapid), and the least change in a comparison of the sitting with the standing posture when the cycles are long (pulse rates slow).

II. Women differ from men as follows:

a. Women's hearts beat faster than men's in all positions studied.

b. The pulse of women does not slow as much as that of men as the position becomes more horizontal.

c. Differences in rate between men and women are greater as the rates compared are more rapid.

d. The systoles of women are uniformly longer than those of men at the same cycle length and position.

e. The systoles of women do not lengthen to the same degree as those of men as the position approaches the horizontal.

f. The greater the difference in the pulse rates of women as compared with men, the less is the difference in the length of the systoles.

g. As cycles lengthen, the systoles of men lengthen more than those of women, and the pulse rates decrease more except in the case of the recumbent position, in which the rates are all very slow and so approach each other.

h. As the position approaches the horizontal, the lengthening of systoles and decrease of pulse rates are in both cases greater for men than women.

It is suggested that the explanation for the differences between the pulse rates and systole lengths of men and women may be that the hearts of women are smaller in proportion to the size of their bodies than those of men. We have found in the literature but few statements concerning the relative size of the hearts of women as compared with those of men of like body size and weight, but those we have seen are in favor of the above conjecture (3), (4).

It has been previously pointed out that in standing, and, though to a less extent, in the sitting position, gravity tends to retard the return of venous blood to the heart, but that this influence is lacking in the recumbent posture, so that the heart has to handle more blood in the latter case, and the systoles are consequently longer. A faster pulse would pump the blood out of the veno-auricular reservoir faster, and would tend to reduce the amount of blood which would have to be pumped per beat. This would be of greater advantage in the case of women if their hearts were smaller relatively to the size of body than those of men, and would offer an explanation of the fact that the pulse rates of women are in general faster than those of men, and that the pulse rate does not slow as much as that of men as the subject assumes a more horizontal position.

One must also recognize the fact that the pulse rates of men and women do not change to the same degree by change of position, the hearts of men slowing more than those of women in all cases. That is, the hearts of women maintain a higher rate to compensate for the larger amounts of blood they have to pump in relation to their size.

The hearts of women compensate for their sex-difference either by a relative increase of rate or a lengthening of the systole. In the standing position at fast rates both the effect of gravity and the shortness of the cycles would tend to make the amount of blood to be pumped per beat very small as compared with other positions and longer cycles, and although the hearts of women would probably have to handle more blood, relatively to their size, than those of men, as indicated by the length of their systoles, the amount of blood to be pumped per beat being comparatively small, it is not necessary for compensation to be brought about by a further quickening of pulse rate. Indeed, this would be disadvantageous, as the pulse rate is already rapid.

As the cycles lengthen, in the standing position, a decrease in the difference between the systole lengths of women and men is accompanied by an increasing difference in the pulse rates of the sexes. The hearts of women, then, compensate for their sex-difference at slow rates by a relative increase of rate, and at fast rates by a relative increase of systole length.

In the sitting position, the phenomenon of compensation, by a quickening of heart rate, exists to a greater degree than in standing. The amount of blood to be handled by the heart is more in the sitting position than in the standing, and the excess of pulse rate of women over men is greater at all times, with the result that the systoles of women not only approach those of men in length, but at very slow rates are even shorter than those of men. This "crossing of the curves" takes place at a higher level in the recumbent position, so that the systoles of women are of the same length as those of men at the "average" cycle lengths, and are distinctly shorter at the slower rates. This is accounted for by the fact that the effect of gravity to shorten the systoles is absent in this posture.

There is less difference between the systole length of women and men, however, in the recumbent position than in either of the others, because the pulse rate of women is so much faster than that of men, owing to the fact that the pulse rates of men have quieted down so much more than those of women.

If like amounts of blood were returning to the heart, a small heart would be enlarged more than a large heart by the entering blood, and the small heart would probably take a longer time to drive the blood out.

The fibers would be lengthened during diastole, would probably contract to their natural length during the early part of systole, and the duration of that phase would depend, probably, under normal conditions, on the distance they would have to contract. The stretching would excite more vigorous contractions (Starling, 5), but it is not certain what effect this would have on the contraction time.

The faster pulse rates of women are accompanied by relatively longer systoles, and it suggests itself that these faster rates constitute a compensatory phenomenon, which shortens the systoles of women.

The longer the systoles the larger the amount of blood which will be pumped per beat, and the greater the work of the heart per beat. A faster rate of pumping would enable the heart to pump the same or even a greater amount of blood per minute, and at the same time lessen the amount of work per beat. The shortening of the cycles would, of course, be accompanied by shorter diastoles, entailing a shortening of the period of rest, nevertheless it is evident that it suffices, otherwise the ordinary rapid rates of women could not be maintained.

It would seem as if the most advantageous adjustment of the contraction period to the period of rest would be that of sleep. The normal

position in sleep is the recumbent, and in this position the systoles would probably be long and the heart would have to pump a large amount of blood per beat, but the diastole would also be long, the heart rate being slow, and the long periods of rest would compensate for the extra work of the heart per beat.

It is interesting to note here that patients with impaired hearts find the recumbent position oppressive, and are more comfortable sitting, because in this position the heart does not have to pump so much blood per beat.

SUMMARY

Supplementing the deductions of a previous paper relative to the causes for the systoles of women being uniformly longer than those of men, we have, in this paper, paid especial attention to the reasons for differences in heart rates of men and women.

An examination of table 1 shows the following differences between the heart rates and systole lengths of men and women: Women's hearts beat uniformly faster than men's and they slow down less under the influence of gravity and position, while their systoles are usually longer and are also less influenced by postural changes.

These facts are accounted for under the supposition that the hearts of women are smaller relative to their size than those of men.

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CONCERNING THE EFFECT OF DIET ON THE CONCENTRATION OF THE NON-PROTEIN SULFUR OF BLOOD

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The effect produced by changes in the protein and carbohydrate content of the blood on the absolute and relative concentration of the various sulfur fractions of the urine has been worked out in considerable detail (1).

Investigations, the results of which have recently been published from this laboratory (2), would indicate that there is present in blood at least three types of sulfur compounds, which appear to possess chemical properties similar to the three sulfur fractions present in urine, i.e., inorganic sulfate precipitated directly by the addition of barium sulfate to the deproteinized blood filtrate, "ethereal" sulfates, precipitated by barium sulfate only after the deproteinized blood filtrate has been boiled with acid, and "neutral" (unoxidized) sulfur, which is transformed into sulfate by treatment with appropriate oxidizing agents. The average of the results obtained by the analysis of the blood of five dogs gave the following figures (3):

	MGM. PER 100 CC. BLOOD	PER CENT OF TOTAL NON-PROTEIN SULFUR
Inorganic sulfate	3.35	34.5
Ethereal sulfate	1.58	16.3
Neutral sulfur	4.77	49.2

As will be seen, the largest fraction of the total non-protein sulfur of dogs' blood (49.2 per cent) is represented by neutral sulfur, the smallest by the ethereal or unstable fraction, with inorganic sulfate occupying an intermediate position, a distribution essentially different from that found in the urine, in which with animals on a high protein diet the inorganic sulfate fraction may account for as much as 90 per cent of the total sulfur. In a fairly large series of determinations of the distribution of these sulfur fractions in human blood and in the blood of animals we have found that the concentrations of the three fractions show rather wide variations in blood taken from different subjects. The cause of these variations is as

yet obscure. As the result of studies made on dogs suffering from uranium nephritis (4) we have found that the level of the inorganic, and to a smaller extent of the neutral sulfur fractions of the blood appear to vary indirectly with the volume of urine, for when diuresis was present the concentration of these fractions became very small, too small in fact for quantitative determination by the method used, whereas in the later stages, when the poison had affected the kidneys to such an extent that the excretion of fluid was reduced to sub-normal volumes the inorganic and neutral sulfur fractions of the blood were increased to as much as three times their initial concentrations. Similar results have also been obtained in a series of observations (an account of which will be published shortly) made in collaboration with Dr. George Herrmann on cases of clinical nephritis. We have also attempted to determine experimentally whether the level of the inorganic sulfate of the blood bears any definite relation to the concentration of this fraction in the urine (5). Dogs fed on weighed constant rations were fed powdered sulfur; and while as a result of this feeding the absolute values of the sulfur fractions of the urine showed marked increase the same result did not invariably occur in the blood. In this paper we wish to present the results obtained in a series of experiments made to determine whether marked variations in the sulfur and protein content of the food produce any effect on the absolute or relative concentration of the sulfur fractions of the blood.

Experimental. Dogs were used as experimental animals. In each case the experiment was divided into two periods and a fore period. During this fore period which usually extended over at least three days the dogs were kept in metabolism cages and were fed the weighed diet low in sulfur (bread and milk, either with or without the addition of sucrose) which was used during period A. During the first experimental period the diet used during the fore period was continued, the urine was collected in 24 hour amounts by catheterization, and 20 to 25 cc. of blood were taken from the heart on the last day. During the second experimental period the animals were fed raw beef; blood and urine were collected as in the first period. In order to render the feces solid and avoid contamination of urine nine grams of fullers' earth which had been boiled with dilute hydrochloric acid, then washed and dried, were administered each day well mixed with the food. In every case one or more dry formed stools were passed each day.

The various non-protein sulfur fractions in blood were determined by the methods recently described by us (2) while the determination of total and inorganic sulfates in the urine were made by the procedure of Folin (6) and total sulfur by the method of Benedict (7).

The urines collected during the several days of each period were poured together and the analyses made on the mixture. The figures for urine

given in the tables and labeled "grams per 24 hours" therefore represent an average figure obtained by dividing the total amount in each fraction by the number of days of the period.

A total of ten experiments has been made.

The diets given in experiments 1 to 5 inclusive were calculated to have a calorific value of 80 to 81 calories per kilo dog per day. Diet A (bread and milk) was calculated to provide 0.02 gram sulfur, and diet B (lean beef) 0.095 gram sulfur per kilo dog per day.

TABLE 1

Dog 7

Female, weight January 25, 9 kgm., March 14, 9.3 kgm.

Food periods A and B, 180 grams bread, 180 cc. milk, 9 grams fuller's earth, calculated to contain 0.116 gram sulfur.

Periods C and D, 378 grams raw beef, 9 grams fuller's earth, calculated to contain 0.685 gram sulfur.

Blood

PERIOD	DATE (1927)	INORGANIC SO_4	ETHEREAL SO_4	NEUTRAL S	
		Mgm. S per 100 cc. blood			
A	February 7	4.86	0.79	5.70	
B	February 21	3.31	0.20	4.42	
C	March 7	3.99	1.0	6.70	
D	March 21	5.64	1.19	6.17	

Urine

		Grams S in 24 hours			Average volume of 24-hour urine
					cc.
A	February 1–7	0.134	0.009	0.035	191
B	February 8–21	0.137	0.0095	0.038	203
C	February 21–March 7	0.345	0.144	0.137	266
D	March 8–21	0.337	0.129	0.142	271

In the last five experiments, which were carried out over much longer periods than were the first five the diets were calculated to have a calorific volume of from 50 to 65 calories per kilo dog.

The amount of carbohydrate and protein in the two rations varied widely, the lean beef being calculated to contain approximately 20 to 22 per cent protein, 10 per cent fat, 60 per cent water and to be essentially free from carbohydrates, while the bread and milk mixture contained approximately 6 per cent protein, 2.5 per cent fat, 29 per cent carbohydrate and 61 per cent water.

The first five experiments were run in periods of 5 to 7 days, and while the changes in the sulfur fractions of the urine were uniform, as with increase in the sulfur (and protein) content of the food there was found a rise in the absolute concentrations of the inorganic, ethereal and neutral sulfur fractions, the results of the analysis of the blood made at the end of each period showed in four experiments a decrease in the inorganic sulfates of the blood after the period of high sulfur high protein diet,

TABLE 2

Dog 10

Female, weight January 25, 10.2 kgm., March 7, 10.9 kgm., March 21, 11 kgm.

Food periods A and B, 204 grams bread, 204 cc. milk, 9 grams fuller's earth, calculated to contain 0.182 gram sulfur.

Periods C and D, 430 grams raw beef, 9 grams fuller's earth, calculated to contain 0.792 gram sulfur.

Blood

PERIOD	DATE (1927)	INORGANIC SO_4	ETHEREAL SO_4	NEUTRAL S	
		Mgm. S per 100 cc. blood			
A	February 7	3.31	0.64	5.08	
B	February 21	3.62	0.19	3.64	
C	March 7	1.74	0.46	4.70	
D	March 21	1.88	0.50	5.96	

Urine

		Grams S per 24 hours			Average volume of 24-hour urine
					cc.
A	February 1–7	0.131	0.008	0.049	293
B	February 8–21	0.134	0.010	0.044	274
C	February 22–March 7	0.412	0.132	0.204	364
D	March 8–21	0.348	0.160	0.195	278

whereas in one experiment there was an increase in this fraction; in three cases the neutral sulfur fraction was increased and in two a decrease was noted.

The results of these five experiments are not presented in detail as it was felt that possibly the irregularity of our figures might be due to the fact that the feeding periods had been too short. We have therefore carried on five additional experiments in which the periods of feeding on each diet were extended to from thirteen to twenty-eight days, each period

being divided into two sub-periods, so that each experiment contains data obtained by four examinations of the blood and urine.

An examination of the results of these longer experiments shows that in every case the sample of blood taken after 7 to 14 days of the high sulfur high nitrogen diet shows a decrease from the initial value in the inorganic sulfate fraction which is followed in most cases by a relative rise, which rise in two cases gives values higher than those obtained on the low protein diet and in three instances stops short of this figure. In every case this fall in organic blood sulfate is coincident with a moderate increase in the volume of urine excreted, which follows the administration of the high protein diet. The ethereal sulfates do not appear to bear this relation to urine volume, as in two experiments they show a decrease and in three an increase. In the case of the neutral sulfur fraction there is an increase in every experiment, which increase is in some cases so small as to lie within the limits of experimental error.

In the interest of space economy we give in detail the figures obtained on only two of the longer experiments, both of which extended over a total period of forty-nine days.

SUMMARY

The concentrations of inorganic, ethereal and neutral sulfur of the blood appear to bear no relation to the relative rate of excretion, or to the concentration of these sulfur fractions in the urine, or to the level of sulfur or nitrogen in the food.

The level of the inorganic blood sulfate is apparently closely related to the volume of urine excreted, but it would appear that the variations occurring in the sulfur fractions found in blood must be due to other factors as yet unknown.

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THE ABSENCE OF INHIBITION AHEAD OF PERISTALTIC RUSHES

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It is almost universally taught today that food goes down the bowel because, according to the "law of the intestine," there is always contraction above and relaxation below a stimulated point (Bayliss and Starling, 1899). Strange to say, this teaching continues in spite of the existence of a number of facts that throw great doubt on its soundness; and actually, one has only to glance at the fecal balls advancing along the colon of a rabbit to see that contraction is sometimes almost as marked below as above a distended region (fig. 1).

Most writers on the subject fail also to note that the discoverers of the law had difficulty in demonstrating it; it was erratic enough in the dog, but in the cat and rabbit it was worse; in order to get the expected reactions the animals had to be purged; the bowel had to be denervated, and its coats traumatized and rendered abnormal by the introduction of balloons. The few men who later attempted to confirm the existence of the law all met with difficulties (Magnus, 1904; Langley and Magnus, 1905; Elliott and Smith, 1904; Cannon, 1912); and others (Alvarez and Starkweather, 1919) were unable to show the desired reaction except on rare occasions.

Cannon (1912) pointed out that the myenteric reflex, as he called it, could not preside over a number of the activities of the stomach and bowel, and a moment's thought should show anyone that it cannot be active for any great part of the time or else food once started down the tract would promptly be carried to the anus, and we would all soon be dead from diarrhea and inanition. That Bayliss and Starling themselves realized that the field of application of the law might be limited is shown by the fact that they qualified their statement with the words: "*if cerebral reflexes be excluded*, excitation at any point," and so forth. Unfortunately this introductory clause was promptly deleted by the textbook writers, who, it now appears, might with greater propriety have added other limiting phrases such as: "in the dog," "after purgation," or "in the presence of balloons."

Long ago it seemed to one of us (Alvarez) that, if any importance is

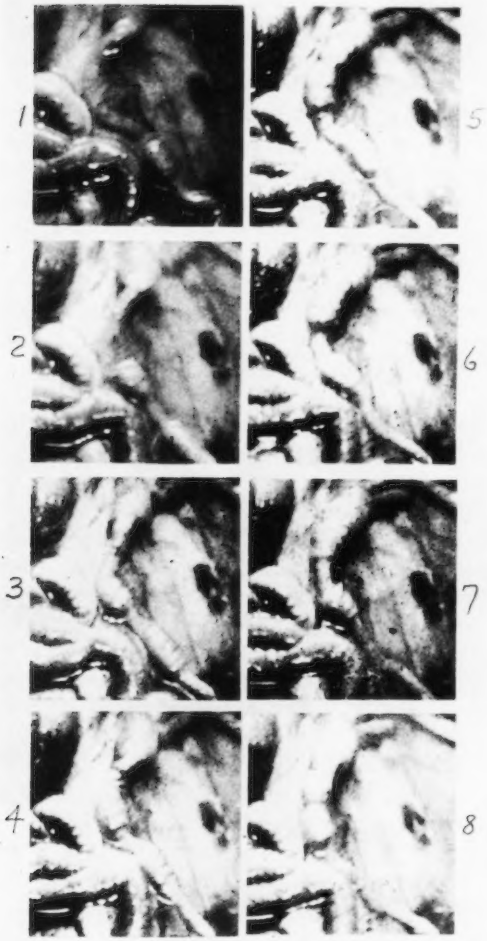

Fig. 1

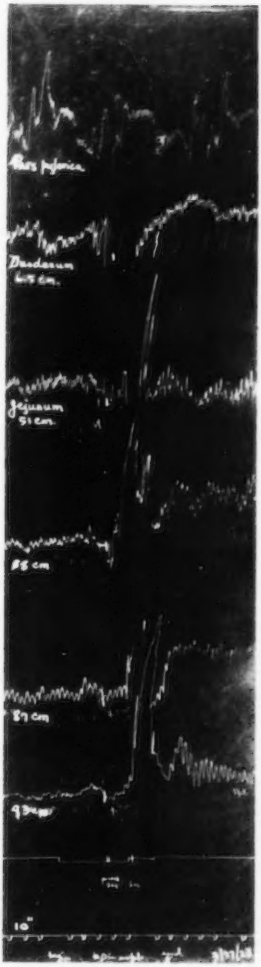

Fig. 2

Fig. 1. Successive pictures from a cinema film of a rabbit's colon showing the progress toward the anus of a relaxed segment between two contracted ones. The interval between pictures was about one second, the anus was just beyond the upper margin.

Fig. 2. Tracings made simultaneously of the activities of the antrum, duodenum and jejunum of a rabbit. The figures in centimeters refer to distances from the pylorus. The passage of a peristaltic rush is depicted. Small white marks show synchronous points.

ever to be attached to the law, its workings should be demonstrable in the case of the peristaltic rushes that normally carry intestinal contents down the bowel, so fifty of these waves were recorded and studied in rabbits opened under salt solution (Alvarez and Mahoney, 1924). As in each animal simultaneous tracings were made of the activities of four or five segments along the length of the bowel (fig. 2), 239 records of the behavior of the gut during the passage of a wave became available for analysis. In 38 per cent of these there was gradual shortening of the muscle for from ten to twenty seconds before the traveling constriction arrived; no preliminary relaxation could be detected in 27 per cent, slight relaxation was observed in 20 per cent, and a definite drop in the level of the record, or an inhibition of one or two rhythmic contractions was observed in 15 per cent. As is shown in figure 2, a depression in the level of the record, suggesting inhibition, could be observed as a wave passed certain recorders and not as it passed others.

After watching the progress of a number of these waves, Alvarez (1924) came to the conclusion that the drop in the level of the record was probably due not to inhibition, but to distention of the bowel by the oncoming column of fluid. He showed that the progress of the wave in the wall of the gut is one phenomenon and the progress of the material in the lumen another, and that the two can easily be dissociated. By cutting the bowel across and putting in lengths of glass tubing he showed that the waves easily jump the gaps, and that descending inhibition is not an essential part of the mechanism of peristalsis.

In order to determine definitely whether the occasional widening of the lumen of the gut that appears before the arrival of the traveling constriction is due to passive distention or to active relaxation, motion pictures were made with the animals opened in the usual way in a bath of warm Locke's solution. In most instances the lower dorsal cord was destroyed in order to eliminate splanchnic inhibitory effects. After the film was made, successive pictures were thrown on a screen, the width of the bowel at three or more points was measured with a pair of dividers, and the distances were plotted as ordinates with successive sixteenths of a second (sixteen pictures were taken each second) as abscissas.

Figure 3 shows how, in this way, we were able to secure curves much like those that had been obtained previously with the help of threads and heart-levers. The space between the two lines connected by a bracket represents the width of the bowel at successive intervals of time. As in the usual graphic records, a drop in the line represents lengthening of the muscle and a rise represents contraction. The small figures drawn on the charts show the appearance of the bowel as the wave or bolus passed, and the positions of the diameters measured. One advantage of the technic is that the bowel is not irritated or pulled on as it is when even the most delicate recorders are attached.

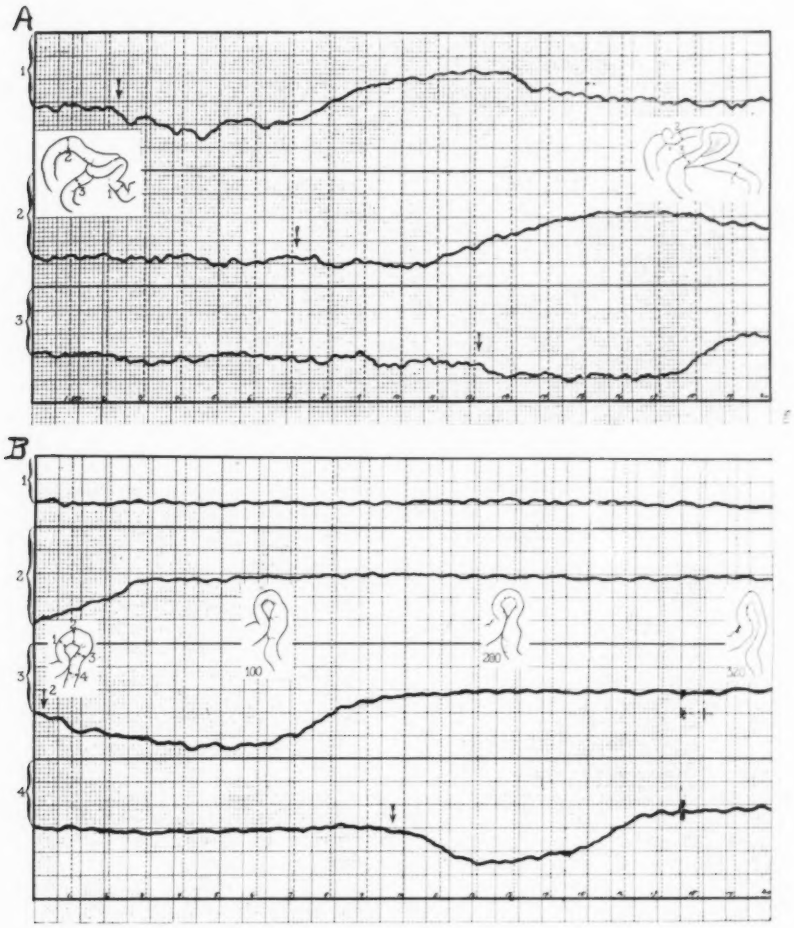

Fig. 3. A, Curves showing the changes in diameter of the small intestine associated with the passage of a peristaltic rush past three points. The measurements were taken from 320 successive pictures in a cinema film. The small inserts show how the loops looked in the first and the 320th pictures. The spaces between the lines connected by brackets represent the varying diameter of the bowel at the three points. A drop in the curve therefore represents a relaxation or stretching and a rise represents shortening of the muscle fibers. The dotted lines represent intervals of one second. The arrow shows when the head of the column of intestinal contents appeared. B, curves similar to those in A, and showing the varying diameter in four places in the colon as a fecal ball passed. The small diagrams show the appearance of the bowel in the second, 100th, 200th and 320th pictures.

The arrow shows when the head of the column of liquid appeared in the pictures, and it will be seen at once that the widening of the lumen

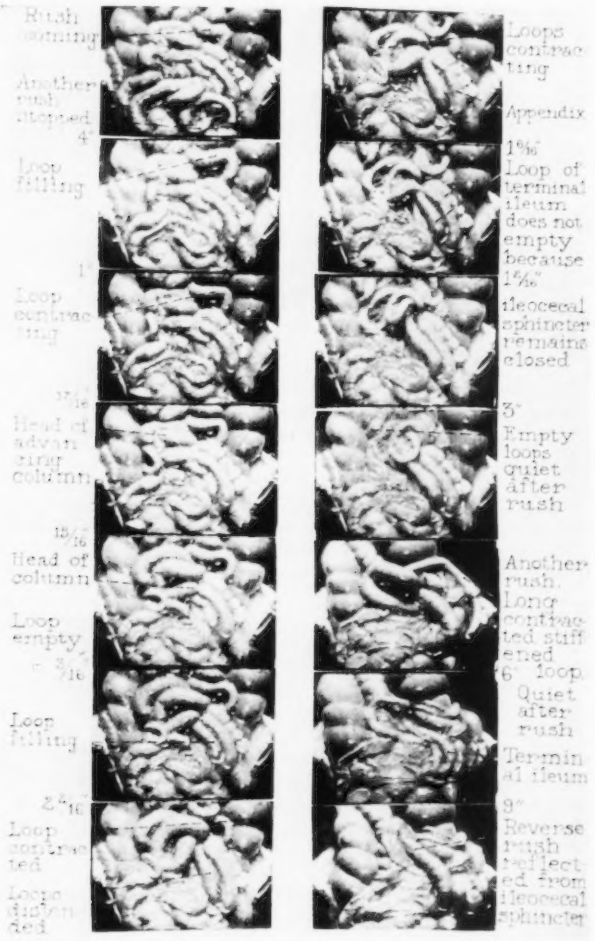

Fig. 4. Pictures taken from a cinema film to show stages in the progress of peristaltic rushes. The last figure on the right shows the head of the column of fluid running backward up the bowel after being reflected from the ileocecal sphincter.

was always due to distention. In some instances there was slight enlargement of the intestine for a fraction of a second before the head of the column of liquid could be made out, but this was probably due to a little

gas moving ahead of the fluid. Certainly there was no sign of relaxation such as could have influenced the downward progress of the wave.

Figure 3B shows the behavior of the colon of a rabbit as a fecal ball passed slowly through it. In this case the stimulus was a spherical bolus much like the balloon used by Bayliss and Starling, but instead of descending inhibition, there is contraction, and the bowel does not widen until the ball is forced into it.

In figure 3A, it should be noted how long it took for a rush to pass a given point and how long the bowel remained contracted afterward. The onrushing fluid is like a long train of cars with an engine behind; the head of the column is many centimeters in advance of the contraction, distending the bowel and overcoming resistance. There is no chance for regurgitation to take place because, by the time the contracted bowel relaxes, the head of the column is well on its way to the iliocecal sphincter. A slight idea of the length of bowel that contracts powerfully in the wake of a rush wave can be observed from the fifth picture in the second column of figure 4.

It is now easy to see why there is often no distention of the bowel as a rush passes a given point. These waves on their way down the bowel pick up fluid here and there; they carry it for a short distance and drop it again much as a wave on Waikiki Beach carries a surf rider for a short distance and then, if he is not skillful, runs on without him. When a rush wave is freighted with a long column of fluid there is much distention of loops ahead, but when it is traveling "light," or when it is running only in the wall of the intestine, there is none. Much depends also on how empty or full the bowel is when the wave arrives; and still more on the way in which the bowel is folded just beyond the point where a recorder is attached. When the loop is full the new material coming down from above may be added so gradually that it is impossible to know just when it arrived, but when the bowel is empty the head of the column can be seen advancing like a foot into a stocking (last picture in fig. 4). The most marked grades of distention are seen when the loop has to untwist a bit before material can pass out of it.

The small irregularities in the record probably have no significance and are due simply to difficulties encountered in measuring the width of the bowel, as recorded in successive pictures. When these pictures are enlarged several times, outlines become hazy and a small error must enter into all the measurements. Careful studies made with small clear-cut markers on the surface of the bowel indicate that the curve of contraction is smooth.

In his classic *Grammar of Science*, Karl Pearson devotes several chapters to combating what he believes is a common and serious misapprehension on the part of scientific men, namely, that we assume that in our technical

field the word law implies legislation and compulsion, much as it does in the world of civics and business, when, actually, it does no such thing. The world is not held in its orbit, as some suppose, by the laws of Newton; we have not yet the slightest idea of the nature or origin of the forces involved. All Newton did was to find a short mathematical formula that sums up and systematizes the host of astronomic observations that in years past have been made on the relative positions and movements of the heavenly bodies. It would seem, therefore, that if relaxation below a stimulated place can seldom be demonstrated in association with contraction above, and if descending inhibition can never be made out ahead of peristaltic rushes, we are not justified in speaking of the "law of the intestine": it is too sweeping a term with which to sum up the results of a few observations made for the most part on purged dogs with vagi and splanchnics cut, and with a foreign body in the lumen of the bowel.

Figure 1 shows a spherical relaxed segment traveling down the lower colon between two contracted segments. Usually such a relaxed region contains a fecal ball, but in this case the animal had been suffering with diarrhea and all that was being carried to the anus was gas. As we have watched the progress of peristaltic rushes down the small bowel we have not felt the need, as some have done, of postulating the presence of a special set of reflex arcs in the myenteric plexus, but when we observe the behavior of the colon in the rabbit we do feel that there must be something peculiar about the neuromuscular mechanism in this region which makes it easy for two contracted areas to travel caudad with a constant interval between them.

SUMMARY

By measuring the diameter of the bowel in successive pictures of a cinema film, exposed during the progress of peristaltic rushes down the bowel of rabbits opened under a bath of salt solution, it has been shown that what looks occasionally like descending inhibition is really distention, due to the advancing column of intestinal contents. Balls of feces pass down the colon of the rabbit in spite of the fact that there is a powerful contraction below as well as above the distended region.

The "law of the intestine" can so rarely be demonstrated that students should no longer be taught that it is the cause of normal peristalsis. It is not denied that descending inhibitions can occur under certain conditions, but these conditions are so narrowly circumscribed that the importance of the phenomenon can hardly be as great as Bayliss and Starling assumed it to be.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

X. Assimilation and Conservation of Bile Pigment, Blood Hemoglobin and Muscle Hemoglobin

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The capacity of the living organism to adapt itself to somewhat abnormal states is a fascinating study and these experiments might fall into a chapter upon this immortal subject. It will be seen that during the stress of a severe anemia the body can conserve in remarkable fashion any blood hemoglobin introduced parenterally. Under similar conditions blood hemoglobin introduced into the gastro-intestinal tract can be utilized to only a very limited degree. It seems probable that *muscle hemoglobin* introduced intravenously can be utilized at least in part to reconstruct new *blood hemoglobin* during severe anemia periods in dogs. We can submit no experimental data to indicate that bile pigment can be absorbed and conserved toward the construction of new blood hemoglobin.

Conservation of protein split products to form new blood hemoglobin is beautifully illustrated by fasting experiments in which we (Whipple, Hooper and Robscheit, 1920) were able to show a considerable increase in the production of blood hemoglobin above control bread diet periods. This new blood hemoglobin could scarcely be formed from anything but the split products of the host protein and with this illustration of careful conservation of protein material capable of being recast into hemoglobin one need not be surprised to find that 90 per cent of hemoglobin introduced intravenously in severe anemia is conserved to build new and much needed blood hemoglobin. We submit an experiment (table 104) to show that a pancreatic digest of hemoglobin when introduced intravenously will be in part conserved for the production of new blood hemoglobin.

We (Whipple, 1922) have discussed elsewhere the question of "circula-

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tion of bile pigment" and reported experiments (Hooper and Whipple, 1917) which gave no evidence for the absorption of bile pigment from the intestine. This work was questioned by Broun, McMaster and Rous (1922) who submitted experimental data to show that bile pigment was absorbed from the intestine and therefore possibly available for formation of new blood hemoglobin. The recent experiments of Blankenhorn (1927) working in McMaster's laboratory controvert the claims of Broun, McMaster and Rous (1922) as do the experiments of Bollman, Sheard and Mann (1926) which give no data in favor of bile pigment absorption from the dog's intestine.

Our experiments given below show no convincing evidence in favor of absorption of bile pigments from the intestine with utilization to form new blood hemoglobin. Moreover, we have long periods of observation on dogs with bile fistulae opening via the renal pelvis which show no evidence that prolonged deviation of bile from the intestinal tract lessens the capacity of the dogs to form new blood hemoglobin on standard diets. These experiments will be reported in the near future.

Methods. The general methods used in these experiments have been described in detail in the first (Whipple and Robscheit-Robbins, 1925) and fifth (Whipple and Robscheit-Robbins, 1927) papers of this series. The *standard bread* used in all these experiments is described as to ingredients and preparation. This bread contains wheat flour, starch, bran, sugar, cod liver oil, canned tomatoes, canned salmon, yeast and a salt mixture. Bread (8) which contains a little salmon was used in all the experiments here recorded. This bread is a complete diet for an adult dog and will maintain a dog in health for long periods of time, if not indefinitely. This ration keeps the hemoglobin production at a very low level, the average being close to 1 to 3 grams hemoglobin per week period over and above the unknown maintenance factor. There are individual differences and some dogs are constantly high in hemoglobin production on the basal ration as well as on other diets. The weights given for cooked material in all tables represent the weight of the substance *after* cooking and the broth is not added unless so indicated in tables.

The *hemoglobin index* in these papers is a figure obtained by dividing the hemoglobin per cent by the red cell hematocrit in per cent. We believe this figure gives information of value as to the saturation of the red cell stroma with hemoglobin.

The dried red cells used in table 107 were prepared from dog blood, the red cells separated by centrifuge, dried at low temperature and powdered in a mortar. Fresh red blood cells used in table 106 were prepared from fresh citrated dogs' blood, the red cells separated by centrifuge and given at once in the food mixture.

Hemoglobin used for intravenous or intraperitoneal injections was

prepared from normal dog blood collected in isotonic sodium oxalate, centrifugalized, red cells washed twice with normal salt, laked with distilled water, the stroma and residual cells removed by centrifuge, the hemoglobin solution made isotonic with some concentrated saline and injected hypodermically at once. The hemoglobin was given in six doses each week but the total shows in the tables.

The preparation of muscle hemoglobin free from blood hemoglobin has been recently described (Whipple and Robscheit-Robbins, 1926). Some of these extracts in spite of every precaution are quite toxic and must be used with care.

The blood hemoglobin digest was prepared from dogs' red cells digested with pancreatin and fresh dog pancreas in an alkaline medium. A little chloroform and toluol were added to prevent bacterial action. The mixture was digested in an incubator at 38°C. for three weeks. The clear supernatant amber fluid was filtered through paper and given carefully intravenously with no clinical reaction.

The Klim used is a market product of evaporated whole milk powder put on the market by the Merrell, Soule Company.

Experimental observations. In the earlier publications of this series we have stressed the importance of a uniform environment for these anemic dogs. It is of the utmost importance that the dogs tolerate this severe anemia (about one-third normal hemoglobin level) without clinical disturbance and with normal appetite and activity. Unless note is made to the contrary these dogs are normal and healthy during these experimental periods.

Our first experiments with parenteral introduction of hemoglobin were concerned with intraperitoneal injections. The solutions of hemoglobin were given slowly through a hypodermic needle introduced carefully. The dogs remained quiet and only occasionally gave some clinical evidence of peritoneal irritation. The weekly total of blood hemoglobin introduced is given in table 101. In each experiment were introduced about 33 grams of dog hemoglobin and about 29 grams recovered in the first experiment (dog 24–25). There is no change noted in blood volume or hemoglobin index.

The second experiment deals with an Eck fistula and in this dog the reaction is the same as in the first experiment. The Eck fistula differs in many respects from the normal dog as regards blood regeneration but this will be taken up in subsequent publications. This Eck fistula shows about 16 grams hemoglobin production in the fore period of 3 weeks; making the same allowance in the after period we arrive at a recovery of 30 grams following the injection of about 33 grams dog hemoglobin.

We can refer to a third published experiment, table 2, paper 1 of this series (Whipple and Robscheit-Robbins, 1925). The dog (19–104) re-

ceived a total of 46 grams dog hemoglobin intraperitoneally during 2 weeks with almost 100 per cent recovery during the second and third weeks of the experiment.

The experiments in table 102 show intravenous injection of dog hemoglobin of varying amounts from a relatively low value of 18 grams to a high total of 55 grams. In all instances we note a very complete con-

TABLE 101

Dog hemoglobin given intraperitoneally

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
Dog 24–25, bull, male, adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 500	83	16.0	914	4,4	0.46	1.97	20.6	41	1.1
Hemoglobin total 20.6 grams, bread 500	85	16.3	942	5,5	0.45	2.05	23.8	49	1.4
Hemoglobin total 12.2 grams, bread 500	98	16.0	874	6,9	0.48	1.97	24.2	48	30.8
Bread 375, salmon 75	100	16.1	884	5,0	0.48	1.97	24.2	48	1.3
Dog 24–90, bull, male, adult—Eck fistula									
Bread 450, Klim 50, salmon 75	90	19.8	1131	4,7	0.51	2.08	23.0	48	1.3
Bread 450, Klim 50, salmon 75	100	19.8	1148	5,4	0.45	2.05	23.8	49	1.3
Bread 450, Klim 50, salmon 75	97	20.3	1157	5,6	0.47	2.21	20.2	45	13.2
Hemoglobin total 8.6 grams, bread 450*	73	20.4	1115	5,7	0.44	2.17	22.9	50	1.6
Hemoglobin total 24.7 grams, bread 450*	83	19.6	1150	6,2	0.49	2.13	24.3	52	20.2
Bread 375, Klim 50, salmon 75	100	20.1	1208	5,6	0.45	2.13	23.2	49	14.2
Bread 375, Klim 50, salmon 75	100	20.3	1125	5,5	0.48	2.21	20.1	44	12.3
Bread 375, Klim 50, salmon 75	98	19.8	1182	4,9	0.46	2.10	21.3	45	1.4

* Klim 50 grams + salmon 75 grams added to each day's ration.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

servation of the injected hemoglobin amounting probably to 90 per cent or even more of the total hemoglobin which reappears in new red blood cells. There is considerable difference in the time interval between the injection and reappearance of the new hemoglobin. There is usually a lag of about a week just as in the case of a favorable diet intake and one may assume this time is required for the fabrication of the new hemoglobin and red cell stroma from various split products. The time interval may

vary considerably more in the case of hemoglobin injection as contrasted with favorable diet (liver) intake. This may indicate that the process of hemoglobin conservation may be somewhat different than the process of new production from diet intake. The first experiment illustrates a maximum delay of two weeks before there were abundant new red cells available from the preceding injection of 29 grams of dog hemoglobin. The usual period of delay reckoned from the start of hemoglobin injection is one week. The second experiment illustrates by contrast a very prompt response to hemoglobin injection. The injection was given in 12 doses of about 4.5 grams each, beginning the middle of the first week and ending the middle of the third week. There was a prompt appearance of the new formed red blood cells in the second week and almost complete output recovery at the end of the third week. There is usually some "carry over" noted in the first or second week following completion of the hemoglobin injections. A similar "carry over" is noted in dogs on favorable diet intake when one sees hemoglobin production in considerable excess in the first or second week of control diet following the favorable diet. There is good evidence that much of this reserve storage responsible for the "carry over" resides in the liver.

The third experiment shows a typical reaction following a short experiment with 18.2 grams hemoglobin given in a single week. In all these three experiments we observe a rise in the hemoglobin index during the period of hemoglobin injection. This might indicate an excess production of hemoglobin as contrasted with stroma but it is not always observed in similar experiments (see table 103).

Two characteristic experiments are given in table 103. The amounts of fresh dog hemoglobin given intravenously are 47 and 30 grams respectively. The injections were started in the middle of the first week indicated and completed in the middle of the third week. There is a lag of about one week before an excess of new hemoglobin is available for bleeding and there is a "carry over" into the first week following the hemoglobin injections. The recovery of the injected hemoglobin is close to 90 per cent, perhaps a little more or less depending on the average production capacity of each dog during control periods as calculated over a long period of time. There are no noteworthy fluctuations of the blood volume or hemoglobin index.

We have observed some rather severe clinical reactions following hemoglobin injections. We have suspected that slight delay in the utilization of the fresh prepared hemoglobin might be the cause. Possibly the distilled water used in laking the corpuscles may be responsible in some instances. At any rate when there is a severe reaction following hemoglobin injection we are apt to observe considerable *delay* in the appearance

of the conserved hemoglobin but no notable decrease in the total amount saved for subsequent production of new hemoglobin. Dogs 18–114 and

TABLE 102

Dog hemoglobin given intravenously

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 18–114, bull, female adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 275, salmon 100, Klim 25	53	13.3	794	3,3	0.70	2.08	22.1	46	1.2
Bread 200, salmon 100, Klim 50	91	13.2	714	3,2	0.71	2.07	21.9	45	1.4
Hemoglobin total 14.7 grams*	80	13.2	704	3,4	0.70	2.11	22.4	47	1.3
Hemoglobin total 14.2 grams*	81	13.4	702	4,3	0.57	2.16	22.9	49	1.4
Bread 200, salmon 100, Klim 50	100	14.1	772	3,4	0.72	2.14	22.8	49	1.4
Bread 200, salmon 100, Klim 50	100	14.1	830	3,8	0.76	2.24	19.5	44	19.7
Bread 200, salmon 100, Klim 50	98	14.7	891	3,7	0.70	2.14	19.5	42	14.9
Bread 200, salmon 100, Klim 50	100	14.3	867	3,1	0.71	2.27	19.4	44	1.3
Dog 24–46, bull, female, adult—splenectomy									
Bread 500, salmon 75	100	19.0	974	4,4	0.58	2.21	22.9	51	1.4
Bread 500, salmon 75	100	19.7	1048	5,3	0.51	2.03	20.0	41	14.7
Hemoglobin total 10.2 grams†	100	20.0	1054	5,2	0.44	2.01	22.6	46	1.3
Hemoglobin total 28.6 grams†	98	20.0	1090	6,0	0.54	2.17	25.2	55	22.7
Hemoglobin total 16.0 grams†	70	19.6	966	4,9	0.56	2.16	20.9	45	32.1
Bread 500, salmon 75	97	19.4	968	4,2	0.55	2.08	22.3	46	1.3
Dog 21–67, bull, male, adult—splenectomy									
Bread 250, salmon 100, Klim 25	100	12.0	658	4,0	0.56	2.00	22.5	45	1.3
Hemoglobin total 18.2 grams‡	100	12.1	628	4,1	0.64	2.14	22.9	49	13.5
Bread 300, salmon 100, Klim 25	88	12.3	605	4,1	0.62	1.96	20.2	40	12.3
Bread 250, salmon 100, Klim 25	93	12.4	605	4,4	0.49	1.91	22.6	43	1.2

* Bread 200, salmon 100, Klim 25.

† Bread 500, salmon 75.

‡ Bread 250, salmon 100, Klim 25.

21–67 in table 102 showed slight clinical reaction to the hemoglobin injection but the other three dogs were not disturbed.

Table 104 gives some evidence to show that *muscle hemoglobin* may be conserved and utilized to form *new red cell hemoglobin* in severe anemia. These experiments are difficult and not wholly satisfactory but the evidence certainly is in favor of utilization of muscle hemoglobin to form blood hemoglobin under stress. The muscle hemoglobin solutions may

TABLE 103

Dog hemoglobin given intravenously

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 25–24, coach, male, adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 375, salmon 100, Klim 25	100	12.4	788	5,0	0.42	1.80	23.4	42	1.2
Bread 375, salmon 75, Klim 15	100	12.2	788	5,1	0.41	1.83	23.0	42	1.2
Hemoglobin total 6.2 grams*	100	12.4	772	5,4	0.42	1.77	25.5	45	1.2
Hemoglobin total 18.2 grams*	100	11.9	710	6,4	0.45	1.75	25.8	45	29.0
Hemoglobin total 22.4 grams*	100	12.6	750	4,9	0.46	1.75	25.8	45	1.4
Bread 375, salmon 75, Klim 25	100	12.7	744	5,8	0.45	1.88	19.9	37	24.4
Bread 375, salmon 75, Klim 25	100	12.7	744	3,8	0.49	1.88	19.9	37	1.1
Dog 25–46, bull, female, adult									
Bread 300, salmon 75	100	10.5	589	3,9	0.40	1.81	17.3	31	1.0
Bread 300, salmon 75	100	10.7	668	4,4	0.43	1.75	22.0	38	1.1
Hemoglobin total 3.7 grams†	93	10.8	618	5,3	0.32	1.76	21.9	38	1.4
Hemoglobin total 11.4 grams†	97	10.7	560	6,6	0.44	1.80	24.1	43	15.7
Hemoglobin total 15.5 grams†	100	11.2	590	5,4	0.40	1.74	25.2	44	1.3
Bread 350, salmon 75, Klim 25	100	11.3	608	6,5	0.45	1.79	22.3	40	13.3
Bread 350, salmon 75, Klim 25	100	11.3	600	4,8	0.47	1.84	24.4	45	1.3

* Bread 375, salmon 75, Klim 15.

† Bread 350, salmon 50.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

be toxic when given in considerable amounts and the dog can tolerate only a relatively small amount of muscle hemoglobin as compared with blood hemoglobin. The threshold of renal excretion is very much lower for muscle hemoglobin as compared with blood hemoglobin so that large doses of muscle hemoglobin will cause a considerable overflow in the

urine. The first experiment of table 104, dog 24–59, shows our maximal dosage of muscle hemoglobin in a considerable series of experiments as this dog could tolerate one gram per day. This dog regenerates more blood hemoglobin per week than the average and this is a constant personal factor observed in certain dogs of our colony. Taking this into consideration and allowing about 12 grams hemoglobin per three-week period as his base line production on the standard diet we observe an output of 10 to 12 grams hemoglobin above this level following the injections of muscle hemoglobin. There is a longer period of delay than is observed in the subsequent control injection of blood hemoglobin in equivalent amounts. But the evidence favors actual *conservation of muscle hemoglobin* to form new *blood hemoglobin*.

The second experiment in table 104 (dog 25–46) is less convincing as the amount of muscle hemoglobin tolerated by this dog is only one-half that given in the preceding experiment. Both the muscle hemoglobin and the blood hemoglobin injections are on the border line of recognition, for the slight variations due to hemoglobin anemia level and bleeding amounts are close to the amounts introduced. The evidence, however, is in favor of some conservation of both muscle hemoglobin and blood hemoglobin in both experiments on this dog 25–46. Six other similar experiments with muscle hemoglobin have been done.

We have tried to concentrate muscle hemoglobin in some way to enable us to use more of it in such experiments but the muscle hemoglobin is denatured and often rendered very toxic.

We report only one experiment with a pancreatic digest of red blood cells given intravenously but it is quite convincing. The original digest contained 54 grams of dog hemoglobin and was digested for three weeks at 38°C. The supernatant fluid was clear amber and non-toxic but there was abundant dark brown residue which was thought to be in part undigested hemoglobin or hematin. The dog showed an increase of about 23 grams of hemoglobin above the control level which we must assume is due to the intravenous digest. That there should be such a considerable conservation is perhaps surprising especially as most if not all of the injected material must have been in the form of small split products or amino acids. We note the same lag in the curve of hemoglobin regeneration as in the feeding and hemoglobin injection experiments. This favors the idea that all introduced hemoglobin may be in large measure broken down before it is resynthesized to form new hemoglobin.

The three experiments in table 106 are pretty uniform and show only a very limited utilization of fresh hemoglobin given by mouth. We observe that only 7 to 10 per cent of the ingested blood hemoglobin appears as excess hemoglobin removed by bleeding. We may expect an average utilization of 10 per cent of ingested hemoglobin to form new blood hemo-

TABLE 104

Muscle hemoglobin and blood hemoglobin intravenously

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–59, bull, male, young adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Muscle hemoglobin total 6.78 grams*	85	16.7	1260	5,0	0.50	1.82	21.0	38	12.1
Muscle hemoglobin total 6.69 grams*	79	16.6	1035	5,2	0.42	1.89	23.1	44	1.3
Bread 400, salmon 100	96	16.4	1022	5,3	0.44	1.90	24.8	47	1.4
Bread 400, salmon 100	100	16.4	1041	5,6	0.44	1.91	25.7	49	1.5
Bread 400, salmon 100	100	16.5	930	5,8	0.45	2.07	25.0	52	12.2
Bread 400, salmon 100	100	16.6	920	6,0	0.43	1.88	23.5	44	12.9
Blood hemoglobin total 7.14 grams†	100	16.6	1068	5,2	0.46	1.95	24.3	47	1.4
Blood hemoglobin total 6.5 grams†	100	16.8	1020	6,6	0.41	1.85	20.9	39	14.7
Bread 450, salmon 100	100	16.8	985	5,6	0.41	1.77	26.2	46	1.3
Bread 450, salmon 100	100	17.1	964	6,9	0.54	1.79	28.3	51	13.5
Bread 450, salmon 100	100	17.4	1025	6,3	0.37	1.83	25.3	46	1.4
Dog 25–46, bull, female, young adult									
Muscle hemoglobin total 3.77 grams‡	68	10.9	612	4,2	0.48	1.68	23.7	40	2.3
Muscle hemoglobin total 2.77 grams‡	82	10.8	582	5,4	0.39	1.88	22.4	42	1.2
Bread 175, salmon 75, Klim 25	88	10.9	543	5,0	0.48	1.91	25.0	48	1.4
Bread 225, salmon 75, Klim 25	100	11.0	600	5,4	0.45	1.86	26.2	49	1.4
Bread 250, salmon 100	100	10.9	582	5,8	0.44	1.76	22.9	40	12.5
Bread 250, salmon 100, Klim 25	100	11.0	595	4,6	0.48	1.86	23.7	44	1.3
Bread 300, salmon 100	98	10.9	606	6,2	0.42	1.77	20.1	35	10.4
Blood hemoglobin total 3.37 grams§	83	10.7	578	5,3	0.40	1.74	24.3	42	1.3
Blood hemoglobin total 3.34 grams§	74	10.8	576	5,8	0.43	1.81	25.9	47	11.2
Bread 275, salmon 100	90	10.6	670	5,3	0.40	1.69	25.4	43	1.2
Bread 275, salmon 100, Klim 25	92	10.7	584	6,1	0.38	1.74	26.9	47	1.4

* Bread 550, salmon 75, daily diet.
† Bread 400, salmon 100, daily diet.
‡ Bread 200, Klim 25, salmon 100, daily diet.
§ Bread 300, salmon 100, daily diet.

globin. This is probably due largely to incomplete digestion of the ingested hemoglobin. Compare this with the 40 per cent utilization of a hemoglobin digest given intravenously where the hydrolysis by pancreatic

enzymes was continued over a long period of time—table 105. In these experiments we note an increase in the hemoglobin index during the first week of red blood cell feeding.

To compare with the fresh red cell feeding we submit experiments with dried red cells and a commercial product—a blood paste.

The dried cells seem to be rather favorable for digestion and a 20 per cent utilization of the ingested hemoglobin is shown. We can refer to two other published experiments with dried red blood cells—table 2, paper I (Whipple and Robscheit-Robbins, 1925) and table 25, paper II (Rob-

TABLE 105

Dog red cell pancreatic digest intravenously

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–59, bull, male, young adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 550, salmon 75	100	14.5	983	5.9	0.35	1.95	21.0	41	1.0
R. B. C. digest total 31.6 grams hemoglobin*	100	14.9	990	5.9	0.40	1.80	25.0	45	2.0
R. B. C. digest total 27.2 grams hemoglobin*	100	15.8	1036	6.0	0.44	1.88	26.1	49	15.2
Bread 550, salmon 75	98	15.6	957	5.8	0.41	1.87	25.7	48	2.1
Bread 550, salmon 75	100	15.9	1022	6.7	0.37	1.86	24.3	45	12.3
Bread 550, salmon 75	96	15.9	1006	5.2	0.42	1.85	24.0	44	1.3

* Bread 500, salmon 75, daily diet.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

scheit-Robbins and Whipple, 1925) of this series. These two experiments show wide differences of total utilization from a minimum of 6 per cent to a maximum of 25 per cent. The blood paste shows a higher utilization—about 24 per cent. We estimated the hemoglobin in this blood paste by the usual colorimetric method which possibly was much too low due to denaturing of hemoglobin by the process of manufacture. It is therefore possible that this blood paste contained much more actual hemoglobin and denatured hemoglobin than our readings would indicate. This might account for the apparent increased utilization of this hemoglobin product. It is obvious that these hemoglobin concentrates have

not the remarkable power to accelerate hemoglobin production which is constantly observed due to a heavy diet of liver or kidney.

TABLE 106

Fresh red blood cells of dog by mouth

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 25–24, bull, coach, male, adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 350, Klim 25, salmon 100	100	12.4	855	3,1	0.55	1.85	18.4	34	0.9
R. B. C. 20 grams hemoglobin, bread 350, salmon 100	100	12.3	807	4,8	0.53	1.99	22.3	44	11.5
R. B. C. 20 grams hemoglobin, bread 350, salmon 100	100	12.5	794	4,6	0.49	1.91	23.6	45	1.2
Bread 375, Klim 25, salmon 100	100	12.6	764	5,0	0.50	1.91	21.9	42	10.1
Bread 375, Klim 25, salmon 100	100	12.5	794	4,3	0.44	1.86	20.5	38	1.2
Dog 18–114, bull, female, adult									
Bread 250, salmon 100, Klim 25	90	14.1	848	3,0	0.88	2.22	22.1	49	1.5
R. B. C. 15 grams hemoglobin, bread 250, salmon 100	100	14.4	847	3,2	0.80	2.31	18.8	43	13.8
R. B. C. 15 grams hemoglobin, bread 250, salmon 100	96	14.5	817	3,2	0.81	2.19	18.9	41	12.3
Bread 275, salmon 100, Klim 25	53	13.3	794	3,3	0.70	2.08	22.1	46	1.2
Bread 200, salmon 100, Klim 50	91	13.2	714	3,2	0.71	2.07	21.9	45	1.4
Dog 19–104, spitz, male, adult									
Bread 225, salmon 100	90	11.8	653	3,6	0.61	2.18	20.4	44	1.3
R. B. C. 10 grams hemoglobin, bread 200, salmon 100	100	11.8	653	4,1	0.63	2.33	20.0	46	13.2
R. B. C. 10 grams hemoglobin, bread 200, salmon 100	100	11.8	647	4,0	0.65	2.17	18.5	40	14.2
Bread 225, salmon 100	98	11.7	616	3,6	0.56	2.13	19.1	41	1.2
Bread 225, salmon 100	90	11.6	654	3,8	0.58	2.19	20.2	44	1.3

We have performed a large number of experiments in which various types of bile feeding have been tested. We have reported other experi-

ments with dried ox and pig bile, table 1, paper I (Whipple and Robscheit-Robbins, 1925) and table 31, paper III (Whipple and Robscheit-Robbins, 1925a) of this series, which are absolutely negative. We have a series of 14 experiments with fresh dog bile as illustrated in table 108.

TABLE 107

Blood paste (commercial) and dried red cells by mouth

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–46, bull, female, adult—splenectomy									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 400, salmon 75	100	18.7	1036	4,1	0.54	2.00	22.3	45	1.3
Blood paste 60 cc. (14.8 grams hemoglobin)*	100	19.2	995	4,6	0.62	2.04	21.4	44	20.2
Blood paste 60 cc. (14.8 grams hemoglobin)*	100	19.4	982	4,3	0.62	2.05	21.3	44	18.5
Bread 400, salmon 75	100	19.0	1041	4,4	0.53	1.96	23.6	46	1.4
Bread 400, salmon 75	100	19.2	1025	4,9	0.53	2.00	18.4	37	17.0
Bread 400, salmon 75	100	19.1	1015	3,9	0.52	2.04	19.8	40	1.2
Dog 18–114, bull, female, adult									
Bread 450	86	13.2	852	4,3	0.75	2.36	27.4	65	1.5
Bread 450	56	12.4	954	3,8	0.60	1.99	22.8	45	17.3
R. B. C. 10 grams, bread 450	88	12.5	909	3,6	0.61	2.00	21.8	44	16.2
R. B. C. 10 grams, bread 450	94	13.1	870	4,5	0.58	2.00	26.0	52	1.3
R. B. C. 10 grams, bread 450	87	13.4	940	4,2	0.58	1.99	24.6	49	15.7
R. B. C. 10 grams, bread 450	85	13.6	918	4,5	0.55	1.92	25.6	49	16.9
Bread 450	54	12.9	846	5,3	0.53	1.99	23.7	47	17.6
Bread 450	58	12.7	778	4,7	0.53	2.00	24.8	50	1.5

* Bread 400, salmon 50 daily diet.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

We have used varying amounts, some given by stomach tube and others in which the bile was mixed with the standard ration before feeding. The experiments in table 108 were all stomach tube feeding of bile so that we can be sure of complete ingestion just prior to feeding. Large amounts of

TABLE 108

Fresh dog bile by mouth

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–22 coach, female, adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bile 100 cc., bread 400, salmon 50	82	13.6	766	5,1	0.42	2.08	20.7	43	1.2
Bile 100 cc., bread 400, salmon 50	80	13.4	732	5,9	0.43	2.13	24.0	51	1.4
Bread 400, salmon 50	100	13.8	800	5,1	0.46	1.99	23.5	47	1.3
Bread 400, salmon 50	80	13.2	650	5,0	0.49	1.92	25.7	49	1.4
Dog 21–67, bull, male, adult—splenectomy									
Onions 150, bread 300, salmon 50	100	11.9	602	6,0	0.51	1.96	19.5	38	17.5
Onions 150, bread 300, salmon 50	100	12.0	613	4,7	0.52	1.95	25.3	49	1.3
Bread 300, salmon 50	100	11.8	576	4,9	0.57	1.96	20.6	40	15.0
Bread 300, salmon 50	100	12.0	621	4,2	0.63	—	—	41	12.4
Bile 100 cc., bread 300, salmon 75	82	11.5	697	4,1	0.52	2.00	21.7	43	1.2
Bile 100 cc., bread 300, salmon 75	89	11.5	622	4,8	0.56	2.04	19.6	40	13.7
Bread 300, salmon 75	93	11.8	684	4,6	0.48	2.08	21.3	44	1.3
Bread 300, salmon 75	86	11.6	608	6,0	0.44	1.90	20.0	38	12.8
Dog 19–104, bull, male, adult									
Bread 250, salmon 100	86	10.9	638	5,0	0.45	2.06	21.8	45	1.3
Bile 75 cc., bread 250, salmon 100	79	11.0	626	5,1	0.49	1.95	25.5	50	1.3
Bile 75 cc., bread 250, salmon 100	85	11.0	550	5,6	0.52	2.13	21.9	47	14.0
Bread 250, salmon 100	83	10.5	627	3,9	0.53	2.07	19.9	41	1.0
Dog 24–26, bull, male, adult									
Bread 400, salmon 50	81	10.9	598	5,2	0.55	1.87	20.5	38	13.8
Bread 350, salmon 75	55	10.3	606	5,5	0.46	2.14	23.4	50	1.3
Bile 100 cc., bread 300, salmon 100	100	10.5	568	4,5	0.62	2.08	21.6	45	13.2
Bile 100 cc., bread 300, salmon 100	71	10.1	552	4,2	0.55	2.17	21.3	46	1.3
Bread 350, salmon 75	90	10.6	578	4,8	0.48	2.01	22.7	46	1.2
Bread 350, salmon 75	94	10.5	636	4,4	0.50	2.07	21.4	44	1.2

bile were given. A large number of experiments are frankly negative. Some experiments might indicate a little stimulus to hemoglobin production—see dog 21–67 and dog 19–104 in table 108. But as one studies these anemia dogs under a great variety of conditions one becomes suspicious of occasional periods of slight hemoglobin production above the usual base line on the standard diet. We cannot control all the factors which influence hemoglobin production and we therefore place no reliance on a reaction which is inconstant and slight. We may say that these reactions come within the physiological maxima and minima and are due to causes unknown. An optimist might choose to explain these minor and inconstant changes as due to bile feeding and the conservation of some pigment material in bile. We would prefer not to be numbered in this group of optimists and report our opinion that bile feeding has no influence on the reconstruction of new red cell hemoglobin.

Discussion. The *site* of this interesting conservation of hemoglobin in the body may properly come up for discussion. Following the work of Doan and Sabin (1926) one would suspect the endothelial phagocytes and indeed these active and numerous cells may actually play an important part in this reaction. Their observations concern fragments of red cells and our hemoglobin solutions of red cells were freed from particles by high speed centrifugalization. When hemoglobin is introduced into the peritoneum one thinks of endothelial phagocytes and mesothelium of the peritoneal lining as concerned in this reaction and also in the transformation of hemoglobin to bile pigment which takes place so promptly (Hooper and Whipple, 1916).

When hemoglobin is given intravenously or intraperitoneally we observe the same curve of hemoglobin regeneration as that following the ingestion of liver or other favorable diet factors. There is a certain lag in the output which may be interpreted in a number of ways. In the case of liver feeding we may assume that time is required to construct the hemoglobin and stroma of the adult red cells which presumably are built from amino acids and other substances derived from the diet intake. In the case of the hemoglobin injection we may choose to believe that the hemoglobin is tucked away somewhere in storage to be utilized as fast as the stroma is fabricated. Or we may choose to believe that the introduced hemoglobin is first broken down to end or intermediate products which are stored and used exactly as though these materials came from the digestive tract. On the whole the evidence seems to favor the last explanation although both mechanisms may be concerned. We have a right to assume that some of these building stones suitable for construction of hemoglobin are stored in the liver and perhaps some of the actual grouping of amino acids into the parent substance of hemoglobin is completed in the liver. The more one studies the degradation of hemoglobin into bile pigments

the more varied becomes the list of cells and tissues which may participate in this reaction (Rich and Bumstead, 1925). Few persons now believe this process is limited to the liver but rather that a great variety of endothelial and phagocytic cells as well as mesothelium of pleura and peritoneum may be concerned. Many parenchyma cells may also have this capacity of breaking down hemoglobin to bile pigment and we may believe that under favorable circumstances or the stress of emergency many of these same cells may be able to take part in the conservation and actual construction of the hemoglobin up to the point where it is utilized in the maturing red cell.

One may recall that bile fistula dogs with anemia will put out less bile pigment than the same dog without anemia. We reported experiments of this type some years ago (Whipple and Hooper, 1917) and suggested that it might indicate a conservation of worn out blood pigment for the production of new blood hemoglobin. These experiments (table 101) give a clear understanding of the remarkable power of conservation exhibited by the body during severe anemia periods. If any more proof was needed this gives the last bit of evidence to explode the old hypothesis that blood hemoglobin freed in the circulation appeared quantitatively in the bile so that the life cycle of the red cell could be calculated from the output of bile pigments per day.

Muscle hemoglobin free from blood hemoglobin has not been frequently introduced into the blood stream and its peculiar reactions deserve further study. Its method of preparation has been recently described (Whipple 1926) as well as some of its chemical peculiarities (Kennedy and Whipple, 1926). In many respects it appears to be quite similar to blood hemoglobin but we observe that it differs sharply when we give it by vein. The same dog can tolerate more than 10 times the dose of blood hemoglobin as compared with muscle hemoglobin. The dog will change some of both muscle hemoglobin and blood hemoglobin into bile pigment (Whipple and Robscheit-Robbins, 1926), but it can conserve blood hemoglobin much more readily than it can muscle hemoglobin (table 104). In this respect the muscle hemoglobin is much like a foreign blood hemoglobin, for example, sheep hemoglobin. We hope to report further experiments dealing with this subject.

SUMMARY

In severe secondary anemia the healthy dog can conserve in remarkable fashion blood hemoglobin introduced intraperitoneally or intravenously. As much as 80 to 90 per cent of the hemoglobin introduced will appear in new formed red cells which are removed and quantitatively measured.

When blood cells are fed one observes the utilization of only a portion of this material; the percentage utilization may vary from 5 to 20 per cent.

When a pancreatic digest of dog red blood cells is given intravenously in severe experimental anemia we may observe a 40 per cent utilization as calculated from the original digest.

Muscle hemoglobin when given intravenously is not well tolerated and its renal threshold is quite low. The amounts given of necessity are very much less than in experiments with blood hemoglobin and it is difficult to be sure of its utilization. It is probable that some of this *muscle hemoglobin* given in severe anemia is utilized to build *new red cell hemoglobin*.

Numerous experiments with fresh dog bile feeding in anemia give no convincing evidence for a "pigment circulation" and bile pigment utilization to form new red cell hemoglobin.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

XI. Iron Effect Separated From Organ Effect in Diet

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At the present time there is confusion in the field of experimental anemia for a variety of reasons. Many workers produce anemia by a deficient diet and study the various factors which contribute to this type of secondary anemia. Under these conditions there is a wide difference of opinion as to whether iron exerts a favorable influence. J. M. D. Scott (1924) records a favorable influence in rats made anemic by a bread and milk diet. Mitchell and Schmidt (1926) likewise make similar observations on rats and report differences depending upon the solubility of the iron salts. Happ (1922) and Simmonds, Becker and McCollum (1927) believe that specific factors influence the onset of these anemias in rats—possibly some of the vitamines. The utilization of iron is believed to be influenced by the intake of certain vitamines. Hart, Steenbock, Elvehjem and Waddell (1925) use rabbits and note that extracts of cabbage and presumably chlorophyll are necessary for the utilization of iron in anemias due to prolonged milk feeding. Williamson and Ets (1925) use rats and a few dogs. They find iron of little value in short anemia experiments and confirm in this respect the earlier experiments of Hooper, Robscheit and Whipple (1920). Interesting experiments on pigeons are reported by Muller (1927).

It would be possible to extend this list of recent experimental work but perhaps enough data have been cited to make the point that *experimental conditions are very different* in these various reports which differ one with the other as to interpretation. Perhaps it is not surprising that rabbits, cattle, guinea pigs and other herbivora should utilize the green vegetables much more efficiently. These green plants make up an all important factor in their diet and the evidence is strong that chlorophyll may contrib-

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ute to the production of hemoglobin. There is always a strong temptation to associate closely chlorophyll and hemoglobin because of the close likeness in chemical structure. Therefore one is tempted to assume that all animals can utilize chlorophyll to build hemoglobin in anemia. This is probably true in the herbivora but as certainly is not true in dogs (Robscheit-Robbins and Whipple, 1925).

It would seem likely that dogs, rats and pigs being omnivorous would be much alike in these anemia reactions and probably more closely resemble the human animal but one must not be too hasty to assume invariably similar reactions to various conditions on the part of these animals and humans. The recent work of Minot and Murphy (1926) will stimulate the necessary wide studies of human clinical anemias under controlled conditions and we can then study various differences between the reaction in human and animal anemias. It need not be stressed that human anemias are all too frequently complicated by a variety of factors—infection, tumors, nephritis, etc.

Another important difference in these varied experimental anemias is the *method of anemia production:* anemia produced by diet, by poisons destroying hemoglobin, by injury of the marrow or lastly by simple hemorrhage. It would seem that simple hemorrhage would give the very simplest form of anemia and for this reason we have employed this method. Dogs will also permit of accurate measurement of the circulating hemoglobin as well as the hemoglobin removed by unit bleedings and in these important features are infinitely more satisfactory than rats.

It is obviously difficult in the present state of our knowledge to make correct interpretation of the various interesting differences in the group of nutritional anemias as compared with less complicated secondary anemias due to bleeding. Nevertheless such attempts are being made with no great benefit to the clarity of the subject as presented in current literature.

Methods. This important subject is covered in the preceding paper and others of this series. The solutions of iron salts were added each day to 50 grams salmon bread which was fed separately just before the daily feeding of the remaining diet mixture.

Experimental observations. We wish to refer to the other published experiments in this series (Whipple and Robscheit-Robbins, 1925, 1927) which give many data on iron feeding (Blaud's pills) and a discussion of the iron content of various organs used in diet mixtures. We have performed other experiments similar to those recorded below but space will not permit the publication of these other data which confirm the published statements.

In table 111 are three experiments in which daily amounts of ferric citrate or chloride corresponding to the iron content of about 300 grams

of fresh spleen were added to the standard anemia ration. It will be seen that the dogs produced an excess of 10 to 25 grams of hemoglobin per 2-week period above control levels due to these small doses of iron. We may

TABLE III

Ferric citrate and chloride in small daily doses

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–49, bull, female, adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
*Ferric citrate 0.04 gram, bread 400, salmon 75	100	15.9	964	4,3	0.55	1.89	25.2	48	1.5
*Ferric citrate 0.04 gram, bread 400, salmon 75	98	16.2	926	5.1	0.55	1.89	22.6	45	28.2
Bread 400, salmon 75	100	16.1	1056	3,4	0.53	2.03	18.0	36	1.1
Bread 400, salmon 75	96	15.9	1025	4,8	0.47	1.79	25.5	46	1.3
Dog 21–67, bull, male, adult—splenectomy									
Bread 350, salmon 100	83	11.9	644	3,8	0.55	1.96	21.4	42	1.1
Bread 350, salmon 100	79	12.1	643	4,7	0.51	2.00	24.0	48	1.4
*Ferric citrate 0.04 gram, bread 300, salmon 100	77	11.5	590	5,5	0.55	1.96	21.8	43	15.5
*Ferric citrate 0.04 gram, bread 300, salmon 100	95	12.0	664	4,1	0.59	1.83	26.2	48	1.4
Bread 250, salmon 100, Klim 25	89	11.8	605	4,6	0.59	1.92	23.5	45	13.2
Bread 250, salmon 100, Klim 25	70	11.6	663	4,0	0.55	1.77	24.8	44	1.3
Dog 24–26, bull, female, adult									
Ferric chloride 0.05 gram†	100	10.8	632			2.17	23.2	50	1.4
Ferric chloride 0.05 gram†	100	10.8	589	5,0	0.50	1.91	26.2	50	1.8
Bread 250, salmon 75, Klim 25	92	10.9	594	5,7	0.50	2.07	23.2	48	15.6
Bread 250, salmon 75, Klim 25	96	10.7	600	4,1	0.54	2.07	21.1	44	1.3
Bread 250, salmon 75, Klim 25	100	10.5	646	4,4	0.49	1.79	23.9	43	1.3

* Equivalent to 300 grams fresh spleen.

† Bread 250, salmon 100, Klim 25 daily diet.

compare these values with those recorded for spleen feeding (Whipple and Robscheit-Robbins, 1927) in which 200 to 300 grams cooked spleen caused an increase of 20 to 50 grams of hemoglobin per 2-week period over

the control level. One may choose to explain about one-half of the favorable influence of spleen feeding as due to the contained iron. Examination of table 113, however, might indicate possible dangers in such interpretation. In table 111 we see no striking changes in the blood volume, color

TABLE 112

Ferric chloride plus pig kidney

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–22 Coach, female, adult									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Ferric chloride 0.06 gram, bread 325, salmon 75	96	12.8	731	4,6	0.49	1.91	23.8	45	1.3
Ferric chloride 0.06 gram, bread 325, salmon 75	100	12.7	708	5,3	0.45	1.80	26.8	48	1.4
Ferric chloride 0.06 gram, kidney 150*..	100	12.8	665	—	—	1.96	26.5	52	56.7
Ferric chloride 0.06 gram, kidney 150*..	100	12.7	678	4,4	0.59	2.00	24.5	49	32.2
Bread 325, salmon 75	100	13.0	713	4.1	0.51	2.04	20.4	42	1.4
Bread 325, salmon 75	100	13.0	765	3,9	0.65	2.03	24.7	50	2.1
Dog 25–24 Bull, male, adult									
Bread 400, salmon 75, Klim 25	100	12.5	725	5,6	0.47	1.89	25.3	48	15.2
Bread 350, meat 50, Klim 50	88	12.0	784	5,4	0.44	1.84	26.0	48	1.2
Ferric chloride 0.06 gram†	100	12.0	786	5,4	0.49	1.83	27.7	51	15.5
Ferric chloride 0.06 gram†	100	11.9	794	4,9	0.46	1.73	26.0	45	12.6
Ferric chloride 0.06 gram, kidney 200‡..	100	12.0	706	6,9	0.43	1.85	27.8	51	30.5
Ferric chloride 0.06 gram, kidney 200‡..	100	12.0	724	6,4	0.54	1.98	26.2	52	34.4
Bread 350, salmon 100, Klim 25	100	12.1	817	5,8	0.50	1.80	18.9	34	22.1
Bread 350, salmon 100, Klim 25	100	12.4	855	3,1	0.55	1.85	18.4	34	0.9

* Bread 225 daily diet.

† Bread 350, Klim 50, meat 35 daily diet.

‡ Bread 250 daily diet.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

or hemoglobin indices. Dog 21–67 had been splenectomized many months previously but we observe no differences between these dogs and normal controls in our anemia experiments. Evidently the compensation for spleen removal is complete and perfect.

TABLE 113

Ferric citrate plus pig liver and kidney

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	R.B.C.	COLOR INDEX	HEMOGLOBIN INDEX	R.B.C. HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED, BLED
Dog 24–46 Bull, female, adult. Splenectomy									
Food, grams per day	*per cent*	*kgm.*	*cc.*	*millions*			*per cent*	*per cent*	*gms.*
Bread 550, salmon 100	63	18.7	1097	5,0	0.43	1.85	23.4	43	1.3
Bread 450, salmon 100*	95	18.9	1021	5,1	0.46	1.86	25.2	47	1.3
Ferric citrate 0.25 gram, bread 400, salmon 75	100	18.8	986	6,4	0.44	—	—	—	16.2
Ferric citrate 0.25 gram, bread 400, salmon 75	100	18.6	1063	5,5	0.59	1.92	29.0	56	32.4
Ferric citrate 0.25 gram, liver 300†	100	18.7	947	5,4	0.66	2.14	27.9	60	28.5
Ferric citrate 0.25 gram, liver 300†	100	19.3	962	5,0	0.82	2.19	22.6	49	76.5
Bread 400, salmon 75	100	18.9	1077	3,9	0.69	2.16	20.6	44	15.6
Bread 350, salmon 75	100	18.6	1003	3,6	0.67	2.17	22.1	48	1.4
Bread 350, salmon 75	100	18.6	1017	4,2	0.63	2.04	20.6	42	12.0
Bread 350, salmon 75	100	18.7	1036	4,1	0.54	2.00	22.3	45	1.3
Dog 24–25 Bull, female, adult									
Bread 550, salmon 75	100	17.9	1133	4,2	0.43	1.98	18.4	36	1.2
Bread 550, salmon 75	100	19.0	1070	4,7	0.44	2.04	20.3	41	1.2
Bread 550, salmon 75	100	19.4	1155	4,7	0.46	1.99	21.5	43	1.4
Ferric citrate 0.2 gram, bread 500, salmon 50	100	19.6	1153	4,8	0.57	2.13	27.4	58	14.3
Ferric citrate 0.2 gram, bread 500, salmon 50	100	20.1	1130	5,6	0.47	1.84	23.0	42	20.1
Ferric citrate 0.2 gram, kidney 300‡	100	20.3	1013	5,9	0.63	2.17	34.6	75	46.2
Ferric citrate 0.2 gram, kidney 300‡	100	20.4	949	5,2	0.72	2.19	26.8	59	45.2
Bread 500, salmon 50	100	20.8	1107	4,2	0.70	2.08	28.6	60	39.4
Bread 500, salmon 50	100	20.7	1200	4,0	0.65	2.26	22.9	52	20.9
Bread 500, salmon 50	100	20.8	1137	4,2	0.65	2.00	18.6	37	18.0
Bread 500, salmon 50	100	20.7	1090	3,9	0.59	2.11	21.7	46	1.5

* Meat extract 5 grams.

† Bread 200 grams daily diet.

‡ Bread 300 grams daily diet.

In table 112 are two experiments in which ferric chloride is given before and during the pig kidney feeding and we observe a very interesting summa-

tion of influences. The first experiment (dog 24–22) shows a fore period with small daily doses of ferric chloride similar to the experiments in table 111. This particular dog illustrates a negative reaction to iron feeding. During the next two weeks of kidney plus iron we see an unusually large output of new hemoglobin and red cells, a total of about 80 grams per 2-week period over the control levels. We also note a distinct increase in hemoglobin and color indices during and just after the combined iron and kidney diet. This may indicate a more rapid production of hemoglobin than of stroma with consequent higher saturation of stroma by the hemoglobin.

The second experiment of table 112 is even more interesting as in this dog the fore period of iron feeding shows some increase of hemoglobin above the control level—about 20 grams per 2-week period as noted in table 111. There is a control bread fore period in which there is a slight "carry over" from a preceding favorable diet period of raw meat. The combined kidney and iron diet gives a large increase of about 60 grams hemoglobin per 2-week period above the control level. Too much blood was removed in the first week of the after period and the hemoglobin level reduced about 10 points below the average. Allowance of 20 grams is made for this in our estimate of 60 grams hemoglobin production. This experiment also shows a definite increase in the hemoglobin and color indices on the last week of combined kidney and iron feeding.

Two experiments in table 113 show that *heavy iron feeding* may have an effect on the hemoglobin production but the subsequent diet periods of liver or kidney plus iron show the expected increase due to liver or kidney plus the increase due to iron in fore periods. This would seem to weaken the argument that some or much of the organ feeding reactions are due to the iron content. It will be noted further that this dosage of iron citrate is about 10 times the amount contained in the liver diet so that a very large excess is present and the addition from the iron contained in the liver is scarcely capable of further influencing hemoglobin regeneration.

The first experiment in table 113—dog 24–46 shows a normal control fore period and a very considerable hemoglobin increase due to large doses of ferric citrate—an output per 2-week period of about 45 grams hemoglobin above the control level. When pig liver, 300 grams per day, is added to the ration rich in ferric citrate we observe a truly remarkable reaction—a summation of influences and an output including "carry over" in the first week after period of about 120 grams hemoglobin per 2-week period over the control level. We note also a definite rise in the hemoglobin index and color index which return to the usual anemia level about the third week of the control after period.

The second experiment in table 113 is practically identical in all respects. The iron citrate exerts a little less influence in the fore period causing an

excess production per 2-week period of about 30 grams hemoglobin above control level, the pig kidney plus heavy iron feeding of the next two weeks causing the *extraordinary total of about 140 grams of hemoglobin* over and above the control level when we include the "carry over" into the first two weeks control after period. There is also an increase in the hemoglobin and color indices during this period of maximum hemoglobin production indicating presumably a more rapid production of hemoglobin than of stroma during these particular periods. There is a decrease in the level of the plasma volume during this period of very active and continued bleeding which is not surprising.

DISCUSSION. It is clear that in severe experimental anemia small amounts of ferric chloride or citrate corresponding to the iron content of spleen fed in usual diets do actually influence the regeneration of new hemoglobin and red cells. One may choose to attribute a part of the spleen diet response to the contained iron.

But it is also clear that a dog placed on a diet with iron salt may or may not respond with an increase in new hemoglobin and red cell output (table 112). This probably depends on an actual iron shortage which may be present or absent. Addition of pig kidney or liver to this ration rich in iron salts will cause the expected increase due to the kidney or liver and *this increase is superimposed on the level of the iron diet.* Both diets influence the production of hemoglobin and these influences are combined with resultant summation (table 113). It is rare in our experience to find entirely separate factors influencing hemoglobin regeneration which can be combined with complete summation of the two effects.

SUMMARY

Small amounts of iron salts added to the diet may or may not cause an increase in blood hemoglobin output above control periods. This may depend on the existence in the experimental animal of an actual iron shortage or deficiency or salt unbalance.

Large or small doses of iron salts may cause an increased production of blood hemoglobin but a continuing diet period of similar iron feeding *plus* a diet of liver or kidney will show the *sum* of the two expected reactions—the reaction of the iron feeding *plus* the reaction expected from the liver or kidney diet (table 113). We record this interesting *summation* of physiological effect of iron salts plus kidney or liver feeding. This lends little support to the argument that much of the favorable influence of organ feeding upon hemoglobin regeneration is due to the iron contained in the liver or kidney.

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BLOOD PRESSURE FOLLOWING ADRENALECTOMY

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In the course of other investigations we have had an opportunity to make some studies on the blood pressure, in rabbits and dogs, following extirpation of both adrenal glands. Our experiments with rabbits have already been published elsewhere (Rogoff and Dominguez, 1924). Two dogs were used for comparing the normal blood pressure with that in rabbits. Later, these dogs were subjected to double adrenalectomy and blood pressure observations continued up till the death of the animals.

Previous reports on the blood pressure of adrenalectomized animals, made by a number of investigators, consist of observations made in acute experiments on anesthetized animals. Trendelenburg (1914) made some studies on cats, utilizing the method employed by Gärtner (compression of the leg and gradual release of pressure until the pallor of the pad begins to disappear) which permits blood pressure measurements to be made without the use of an anesthetic. He removed the adrenal glands in one- and two-stage operations and permitted the animals to recover from the immediate effects of the operation before making the blood pressure observations. The blood pressure, after double adrenalectomy, remained normal for twelve hours or longer, only sinking shortly before death. While the method employed by Trendelenburg permitted blood pressure measurements to be made in non-anesthetized cats, his animals did not survive long enough to have fully recovered from the effects of the operation for removal of the adrenals.

Anesthesia, operative procedures and other conditions capable of influencing the blood pressure render the above mentioned acute experiments of little value. Nevertheless, the better ones among them show that no significant alteration of the pressure occurs as the result of acute suppression of epinephrin secretion from the adrenal glands. This is to be expected in view of the well-established fact that epinephrin secretion from the adrenals is not indispensable for life and health (Stewart and Rogoff, 1917, 1919). It is sometimes argued that the extracapsular chromaffin tissue may suffice to furnish the necessary epinephrin in the absence of the adrenal secretion, but substantial evidence that this tissue secretes epinephrin is lacking. Its presence does not suffice to prevent death when most or all of the cortical adrenal tissue is removed, nor does it prevent the terminal fall of blood pressure in animals dying of adrenal insufficiency. The low blood pressure observed in Addison's disease, if primarily due to disturbed adrenal function, is more probably associated with deficiency of cortical function than with the epinephrin secretion.

Since acute suppression of adrenal function is apparently without effect upon the blood pressure, it is of interest to determine whether or not the

pressure is altered in adrenalectomized animals that have fully recovered from the operation and are surviving a relatively long time. We found no significant change in the blood pressure, up to the time when the animal was obviously moribund, in 19 rabbits that were subjected to bilateral extirpation of the adrenal glands (Rogoff and Dominguez, 1924). Of these, 3 died in the first week (4, 6, 7 days), 8 in the second week (8, 8, 10, 10, 11, 11, 12, 12 days), 3 in the third week (15, 16, 16 days) and 2 in the fourth week (22, 26 days). One survived 89 days and two about 18 months. During the course of the observations, one of the longest survivors was mated and delivered a litter of 4 (dead) rabbits; at autopsy an accessory cortical adrenal body was found in this animal.

Observations on dogs. The blood pressure was measured, in the dogs as in the rabbits, by Van Leersum's (carotid loop) method. Details of the procedure (on rabbits) are described elsewhere by one of us (Dominguez, 1924). Briefly stated, the method consists of transposing a carotid artery into a flap of skin so that it forms a loop on the neck of the animal. Measurements are made by adjusting a small cuff around the loop and inflating it to compress the artery. The cuff is connected with a mercury manometer, and the measurements can be made by auscultation or palpation of the artery beyond the cuff.

Both dogs were non-pregnant females. The adrenal glands were excised in two operations, an interval of about six weeks intervening between the removal of the right and left glands. Blood pressure measurements were recorded before, during and following the removal of each adrenal. One animal survived nearly twelve days, the other nearly thirty-seven days. The blood pressure observations were made daily, under relatively constant conditions and always by the same observer. In neither animal did the blood pressure fall below the levels observed before adrenalectomy, until the dog developed the usual symptoms that precede the moribund state.

Condensed protocol. Dog, female; record number 120–5.

November 25, 1925, prepared left "carotid loop" (thyroid artery ligated). December 22, 1925, to February 1, 1926, systolic blood pressure measurements made by palpation, thereafter systolic and diastolic measurements were auscultatory. April 30, skin of loop is inflamed (under surface); blood pressure measurements temporarily discontinued. June 15, weight 10.5 kgm.; right adrenal excised under ether anesthesia (weight 0.680 gram); loop healing well. June 25, resumed blood pressure measurements. July 9 to 29, measurements discontinued (owing to illness of observer). During this period the animal was in rut.

July 31, weight 11.1 kgm.; left adrenal excised under ether anesthesia (weight 0.850 gram). During the operation systolic pressure alone was measured (by palpation). 11:12 am., surgical anesthesia, blood pressure 152–140 mm. 11:14 a.m., 142–143 mm. 11:15 a.m., incision made; blood pressure 128–130 mm. 11:21 a.m., pulse very feeble, rate 152; blood pressure about 80 mm. 11:23 a.m., adrenal removed; pulse feeble. 11:25 a.m., wound closed; pulse rate 128. 4:30 p.m., systolic blood pressure

180–190 mm. (verified by a number of auscultatory observations); pulse rate 88. Post-operative recovery very good but animal is not yet in usual playful mood.

August 1, excellent condition. August 2, during observation she was annoyed by flies and became excited; pulse rate went up to 200 and the pressure rose from 135 to 160–170 mm. August 17, up to the present she has been in excellent condition. This morning she vomited a small amount of frothy fluid containing a live worm. At noon she ate a good meal. In the afternoon she vomited a small amount, otherwise appears to be in excellent condition. August 22, condition very good; played actively for a long while. August 31, condition unchanged; ate large portion of meat. September 1, seems less active; not as playful as heretofore and runs about less lively than usual. Not eager for food but ate her entire meal in the course of the afternoon. September 2, quiet, not playful; ate entire meal, little by little, during the day. September 3, condition about the same as yesterday; ate her meal of meat but did not care for the bones. September 4, seems apathetic; ate less than half of her meal of meat. In the afternoon she vomited some mucus and partly digested meat. September 5, 1:00 p.m., asthenic, body temperature subnormal; ate none of her meal; passed small amount of tarry feces. Systolic blood pressure 100–110 mm., diastolic below 40 mm. Hg. 5:00 p.m., passed some more tarry feces; is quite asthenic; pulse rate, 34. 8:45 p.m., condition poor; pulse rate, 34; vomited bile-stained mucus; died during the night.

September 6. Autopsy, 10:00 a.m. Subcutaneous fat in usual amount. Thymus large and fleshy. Thyroids normal in appearance, left gland larger than the right and parathyroid in left lobe reddish (the left thyroid artery was ligated when the loop was made). Heart and aorta normal. Carotid in loop normal. Striae in iliacs (normal). Lungs normal. Liver, spleen and kidneys moderately congested. Pancreas congested. Stomach contained bile and blood-stained fluid, mucosa hemorrhagic; small clots and four small erosions in pylorus. Duodenum, contents bile and blood-stained, mucosa moderately congested, small erosion near orifices of pancreatic ducts. Jejunum, contents same as duodenum, becoming more bloody lower down; mucosa moderately congested. Ileum, contents bloody, especially in the lower portion; mucosa congested in upper portion, quite hemorrhagic lower down. Large bowel contained some tarry fecal matter; mucosa and that of the rectum moderately congested. Uterus thick and boggy. Ovaries, small, containing yellowish bodies.

The long period of survival of this animal is explained by the fact that the second adrenal was excised toward the end of the period of rut. It has been shown (Rogoff and Stewart, 1927) that pregnancy and rut exert a protective influence in dogs deprived of both adrenal glands. The blood pressure observations, on this animal, are illustrated in figure 1. Measurements were not made during the operation for removal of the first adrenal because at that time the skin of the carotid loop was somewhat inflamed. During the second adrenalectomy the blood pressure fell considerably when the gland was being isolated for removal. Excessive anesthesia may have been partly responsible for this, but what is probably of greater importance, in explaining the fall of pressure, is that in this case the gland was in very intimate contact with the neighboring nerve structures. However, the animal made an excellent post-operative recovery and within a short time the blood pressure returned to normal.

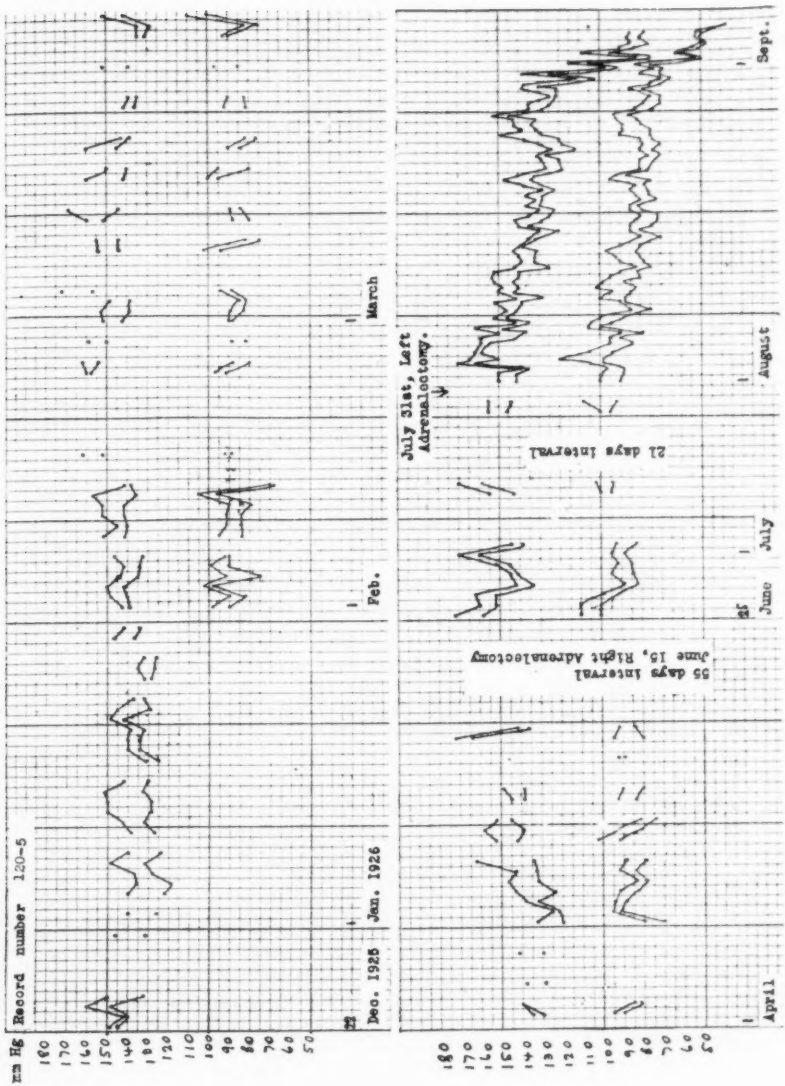
mm Hg
Record number 120-5
180
170
160
150
140
130
120
110
100
90
80
70
60
50
Dec. 1925
Jan. 1926
Feb.
March
April
55 days Interval
June 15, Right Adrenalectomy
25
June
July
21 days Interval
July 31st, Left Adrenalectomy.
August
Sept.

Fig. 1

Superficial inspection of the blood pressure curve in figure 1 may lead to the impression that following the removal of the second adrenal the pressure gradually declined. This, however, is mainly due to the fact that the first few observations after the operation were in the highest levels recorded. On closer inspection, it will be seen that the fluctuations in the balance of the curve were within the same range of pressures observed prior to the excision of the first adrenal, e.g., during January and the first part of April. The first significant fall of systolic blood pressure occurred about six days and of the diastolic pressure about five days preceding the death of the animal, i.e., about a month after extirpation of the second adrenal.

Condensed protocol. Dog, female; record number 120–6.

January 5, 1926, prepared left "carotid loop" (thyroid artery not ligated). May 11, blood pressure measurements, by auscultation and palpation, begun. June 12, between sixth and seventh observations she was disturbed by a fly; the systolic blood pressure rose from 140 to 150 mm., the diastolic from 96 to 105 mm. Hg. June 14, dog panting, room temperature 29.2°C. during the period of observation.

June 15, weight 8.6 kgm.; right adrenal excised under ether anesthesia (weight 0.720 gram). During the operation only systolic measurements were made. 2:17 p.m., incision made; successive blood pressure readings, 147, 148, 152, 154 and 156 mm. Hg. 2:21 p.m., 172 and 172 mm. Hg. 2:24 p.m., 182, 174, 175 and 175 mm. Hg. Adrenal removed. 2:29 to 2:36 p.m., 149, 153, 138, 152, 159, 148, 146, 144, 144, 156, 158, 154 and 156 mm. Hg. 2:36 to 2:40 p.m., 148, 144, 142, 142, 145, 140, and 140 mm. Hg. Wound closed. 143, 143, 145, 150 and 148 mm. Hg. 4:15 p.m., 174, 175, 174 and 174 mm. Hg.

June 16, pulse rate somewhat irregular during measurements. June 18 to July 29, blood pressure measurements temporarily discontinued to permit healing of slight ulceration of the skin on under surface of the loop. July 31, weight 8.3 kgm.; left adrenal excised under ether anesthesia (weight 0.770 gram). 10:37 a.m., surgical anesthesia; blood pressure readings 208, 200 and 195 mm. Hg. 10:42 a.m., incision made; 185, 182, and 188 mm. Hg. 10:43 a.m., 202, 198 and 198 mm. Hg. 10:46 a.m., 200 mm., adrenal massaged, 190 and 190 mm. Hg. 10:47 a.m., 188, 188 and 185 mm. Hg. 10:49 a.m., 168, 170 and 178 mm. Hg. 10:51 a.m., adrenal removed, 158, 156 and 150 mm. Hg. 10:52 a.m., 160 and 158 mm. Hg. 10:54 a.m., 160, 170 and 176 mm. Hg. 10:56 a.m., 158 mm., more ether administered, 164 mm. Hg, wound closed. 10:57 to 11:00 a.m., 150, 158 and 144 mm. Hg. 4:40 p.m., 162, 170 and 170 mm. Hg.

August 1, condition excellent. August 2, room warm, temperature 28.3°C. during blood pressure observations. August 6, condition, up to the present, very good; eating well and behaving normally. August 7, beginning asthenia and anorexia; blood pressure falling; she happened to be irritated by a fly and the blood pressure, which was already low, mounted from 84 to 98 mm. Hg (systolic) and from 60 to 68 mm. Hg. (diastolic). August 8, asthenic; total anorexia. August 9, has developed clonic twitching of the head. August 10, total anorexia; clonus of head less pronounced; asthenic. August 11, total anorexia and marked asthenia; vomited bile and mucus; clonus of head no longer present; died during the night.

August 12. Autopsy. Subcutaneous fat abundant. Thyroids normal. Thymus, small petechiae in lower portion. Lungs and heart normal. Kidneys normal in size; medulla congested; capsule strips with difficulty and tears renal substance; on section shows conspicuous yellowish straight tubules; glomeruli not visible. Uterus

normal. Left ovary, one yellow body in upper pole; right ovary, a number of yellow bodies. Stomach contains bile; mucosa congested. Entire small intestine contains blood and the mucosa is markedly congested, increasing from the lower portion of the duodenum downward to the cecum. Cecum and appendix, mucosa intensely congested. Colon and rectum less congestion. Pancreas congested. Liver slightly congested.

The blood pressure measurements, made on this dog, are recorded in table 1, giving the minimum and maximum pressure in each set of observations. As a routine, each set of observations, in both animals, consisted of ten successive measurements, the average, in the table, representing the arithmetical mean of the ten measurements. No reading was discarded. During the operation for extirpation of the adrenal gland the tracheal respiratory sounds in the anesthetised animal rendered auscultatory observations impossible and measurements were made by palpation only.

No significant alteration in the blood pressure, that might have been attributed entirely or chiefly to the loss of the adrenal, occurred during the operation for removal of the first or second gland. Just before excision of the second adrenal the gland was purposely massaged, with the fingers, to see if this would cause a rise in blood pressure but none was observed. The pressure was somewhat high at the time, however, and it is probable that with a lower pressure an effect might have been obtained, since it is known that massage of the adrenals can liberate sufficient epinephrin to cause a rise in blood pressure (Stewart, Rogoff and Gibson, 1916).

Following the removal of the second adrenal there was no change in the blood pressure during the period that the animal remained in good health, i.e., for about six days. It began to decline with the onset of anorexia and continued to fall as the terminal symptoms developed. The clonus of the head that was present for about three days preceding death rendered the blood pressure measurements rather difficult and the diastolic (auscultatory) observations somewhat unreliable or impossible. When the clonic twitching was not present, it always recurred as soon as the carotid was occluded to make a measurement.

These observations indicate that in dogs, as in rabbits, extirpation of the adrenal glands does not result in any significant alteration of blood pressure during the period of good health. This period, in dogs, may be a week or longer in males, and in non-pregnant females that are not in rut (Rogoff and Stewart, 1926), permitting observations to be made when the animal has completely recovered from the effects of anesthesia and from the surgical procedure. Decline of the blood pressure occurs as a terminal event, associated with other symptoms that have been shown to occur in dogs dying of adrenal insufficiency. It will not be profitable to speculate regarding the primary cause of this terminal fall of pressure,

TABLE 1

Blood pressure observations, dog, record number 120–6

DATE	TIME	BLOOD PRESSURE						PULSE RATE
		Systolic			Diastolic			
		Maximum	Minimum	Average	Maximum	Minimum	Average	
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*per minute*
May 11	10:00 a.m.	162	150	155.0	110	98	103.4	160
May 19	4:00 p.m.	162	152	156.9	112	93	103.5	128
May 20	5:30 p.m.	179	150	164.5	100	93	96.5	160
May 23	1:00 p.m.	152	138	142.5	98	88	92.1	144
May 26	4:30 p.m.	153	148	149.9	103	96	100.1	104
June 9	4:00 p.m.	145	135	139.2	105	92	97.3	144
June 10	5:00 p.m.	145	128	136.5	90	84	88.3	136
June 12	2:00 p.m.	150	139	143.9	105	95	97.2	144
June 14	4:20 p.m.	129	124	125.8	86	75	79.7	136
June 15	2:15 p.m.	Right adrenal extirpated						
	4:15 p.m.	175	174	174.2	125	123	124.0	124
June 16	10:00 a.m.	158	142	150.0	110	100	103.9	120
	5:40 p.m.	162	155	158.0	106	102	105.4	104
June 17	10:00 a.m.	156	152	154.5	111	110	110.1	88
	5:20 p.m.	147	140	142.8	105	98	101.0	86
June 18	10:40 a.m.	145	140	142.4	105	92	97.7	88
July 29	4:45 p.m.	146	144	144.9	112	100	103.3	152
July 30	5:36 p.m.	146	139	142.0	110	102	104.4	120
July 31	10:42 a.m.	Left adrenal extirpated						
	4:40 p.m.	170	162	167.3	130	130	130.0	112
August 1	2:20 p.m.	148	142	143.6	120	115	117.0	104
August 2	10:15 a.m.	162	158	160.8	135	120	124.1	108
	5:20 p.m.	150	142	145.0	100	92	96.2	88
August 3	9:55 a.m.	153	146	149.2	117	110	113.0	96
	5:30 p.m.	152	140	147.6	130	115	118.0	104
August 4	10:15 a.m.	140	120	131.1	110	90	100.8	128
	5:20 p.m.	148	140	142.9	110	94	102.7	112
August 5	10:15 a.m.	140	122	132.8	110	90	98.3	128
	5:15 p.m.	132	124	129.9	104	94	98.9	128
August 6	10:30 a.m.	121	102	112.6	87	76	82.9	128
	4:35 p.m.	110	95	101.8	82	78	80.6	152
August 7	10:40 a.m.	98	76	87.0	70	58	63.5	140
	4:10 p.m.	92	75	85.0	70	54	61.1	148
August 8	12:30 p.m.	100	92	95.2	68	60	63.5	120
August 9	4:35 p.m.	103	88	99.8				136
August 10	9:40 a.m.	89	70	79.2				136
	4:20 p.m.	90	78	84.1	53	50	51.0	128
August 11	10:00 a.m.	98	88	91.5				124
	3:50 p.m.	77	70	72.7				120

but it may be assumed that suppressed epinephrin secretion has little or nothing to do with it, and that it is associated with the phenomena that result from loss of cortical adrenal function.

SUMMARY

Blood pressure measurements were made before, during and following adrenalectomy, in dogs, by Van Leersum's (carotid loop) method which permits observations to be made on non-anesthetized animals. Nearly all the observations included systolic and diastolic measurements.

Observations, made daily, on one dog surviving double adrenalectomy about twelve days and another about thirty-seven days, showed no significant change in blood pressure during the period of good health of the animals. A decided and progressive fall of pressure occurred a few days before death, with the onset of the terminal symptoms. This is in accord with our earlier studies made on rabbits.

The dog that survived nearly thirty-seven days was operated for removal of the second adrenal toward the end of the period of rut.

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STUDIES IN THE METABOLISM OF MUSCLE

I. The Respiratory Quotient of Resting Muscle[1]

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Evidence concerning the source of energy of resting muscle leads to different conclusions according to which of two general methods of study is used. Observation of excised muscle indicates that carbohydrates exclusively supply the energy necessary for the maintenance of normal resting conditions. On the other hand, the study of the whole organism makes for the possibility that not only carbohydrates but also non-carbohydrate foodstuffs are so used. A solution of this contradiction is attempted because of its importance for the understanding both of the physiologic processes of metabolism of muscle and the pathologic phenomena of diabetes.

Meyerhof (1) studied excised frog muscle and showed that under anaerobic conditions lactic acid is formed from glycogen. This lactic acid later disappears in the presence of oxygen, three-quarters of it reappearing as glycogen. The amount of oxygen consumed is practically that required to combine with one-quarter of the lactic acid or its equivalent in some other carbohydrate form which thus seems to be oxidized in order to reconvert the remaining three-quarters into glycogen. The final proof that lactic acid or some other carbohydrate is the only foodstuff oxidized in excised muscle is found in Meyerhof's determination of a respiratory quotient of unity.

Meyerhof and Himwich (2) obtained similar results for excised mammalian (rat) muscle. Here, too, glycogen is the source of lactic acid. Only a portion of the lactic acid formed appears to be oxidized and the respiratory quotient is 1. These observations received further confirmation when Burn and Dale (3) noted that the spinal cat, eviscerated and skinned, hence essentially a muscle preparation, removed sugar rapidly from the blood and had a respiratory quotient of 1. These findings were unchanged even when their cats had been depancreatized three days before the

[1] A preliminary report of this paper was read at the meeting of the American Physiological Society at Cleveland on December 29, 1925 (see This Journal, 1926, lxxvi, 188).

observations. This is in agreement with the previous work of Parnas (4), who found no difference in the metabolism of the excised muscle of normal and of depancreatized frogs. Thus, experiments on excised muscle, both of amphibians and mammals, seem to show that only carbohydrates are oxidized in that tissue. An exception is the work of Takane (5) who, in four observations made in Meyerhof's laboratory on strips of rat diaphragm suspended in serum, found respiratory quotients respectively of 0.83, 0.90, 0.66 and 0.75, averaging 0.79.

Though with this exception all these results on excised muscle are consistent with each other, they are not so with the findings obtained in the intact organism. It is well known that when fat is metabolized in large amounts, as occurs in diets high in fats, in starvation or in diabetes the respiratory quotient may be close to 0.7. Since muscle forms about two-fifths of the soft tissues of the body by weight, it seems surprising that the respiratory quotient of muscle should be very different from that of the entire body. Doisy and Beckmann (6), taking arterial and venous blood under local anesthesia from the femoral vessels of dogs, and therefore working in preparations composed of muscle, skin and bone, published seventeen respiratory quotients fourteen of which varied within normal limits. Here, then, are data suggesting that the respiratory quotient of muscle is not always 1.

There is thus evidence for two contradictory points of view in regard to the character of muscle metabolism. Former observations have been made either on excised muscle bathed in a medium possibly lacking certain substances necessary for normal metabolism or on preparations not exclusively composed of muscle. Hence we undertook the redetermination of the respiratory quotient of isolated muscle which is still part of the body and subject to its regulations.

METHODS. *a. Preparation of the animals.* In an attempt to get a perfectly quiet animal and yet to avoid the use of an anesthetic which might change the metabolism, three procedures were employed: *a*, three dogs were trained to lie on a dog board while the femoral vessels were exposed under 2 per cent novocaine and venous and arterial samples were taken; *b*, six dogs, fasted three to seven days, were decerebrated by Schmidt's (7) method, and blood samples were taken after an interval from the femoral vessels; *c*, ten dogs, fasted four to thirteen days, were first decerebrated and then the blood supply to the gastrocnemius and flexor digitorum sublimis muscle was isolated by a technique developed by Castle and Ray (8). (See fig. 1.) Decerebration caused little hemorrhage, and gave in nearly all the animals quiet breathing of uniform depth. The satisfactory respiratory quotients of the expired air testify further that the method does not produce grossly abnormal respiratory changes.

b. Isolation of the muscles. When the dog had been decerebrated an

incision was made through the skin from the groin to the ankle in the right leg. Identifying and elevating the femoral artery in the groin, all branches were cut between ligatures in order peripherally as far as

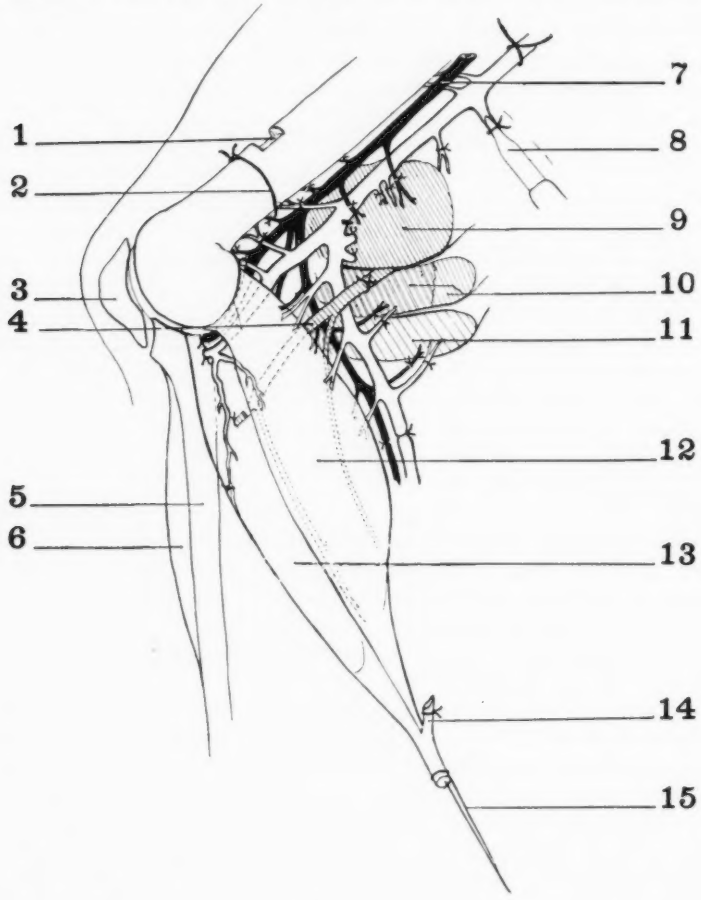

Fig. 1. Diagram showing the relations of important structures in the dissection for the isolation of the circulation to the gastrocnemius and flexor digitorum sublimis muscles of the right leg of the dog. *1*, Notch in femur shaft; *2*, ligature including vestiges of adductor insertion; *3*, patella; *4*, sciatic nerve; *5*, tibia; *6*, anterior tibial muscle; *7*, femoral artery; *8*, cannula in branch of femoral vein for measuring rates of blood flow; *9*, adductor magnus muscle; *10*, semimembranosus and semitendinosus muscles; *11*, biceps femoris muscle; *12*, gastrocnemius muscle; *13*, flexor digitorum sublimis muscle; *14*, insertion of biceps femoris muscle; *15*, string on tendo achillis.

the head of the gastrocnemius, thus avoiding all but trifling bleeding even in dividing the adductor muscles. The dissection was carried along the mesial border of the gastrocnemius, leaving undivided only those branches of the artery and vein that entered the muscles to be isolated. The insertion of the biceps femoris muscle was cut and tied; and then by blunt dissection a plane of cleavage was established between the gastrocnemius group and the muscles on the posterior aspect of the tibia, with exposure, ligation, and division from below upwards of the femoral nerve, the plantar branch of the saphenous artery with its vena comes, several small veins beneath and lateral to the muscle, and the various continuations of the sciatic nerve appearing after penetrating or passing beneath the gastrocnemius. Finally, the popliteal artery and vein were ligated, together with certain small branches of each which passed to the capsule of the knee joint and the fat in the popliteal space.

The leg was then disarticulated at the knee, any remaining soft parts preventing the complete amputation were divided, and all bleeding was carefully controlled. Reflection of the patella and the attached extensor muscles left the lower end of the femur shaft exposed and devoid of all tissues except the origin of the gastrocnemius and a vestige of still undivided adductor insertion. Following the method of Drinker (9), the femur shaft was then deeply notched about two inches from its lower extremity, and the marrow cavity peripheral to the opening packed tightly with vaseline-soaked cotton. With a tight ligature including the shaft of the femur and the remaining adductor insertion the isolation of the muscle was completed. (See fig. 1.)

Injections of India ink following perfusion with physiological saline showed that the circulation of the muscle was not demonstrably disturbed and that its isolation was practically complete. In the fatty tissue surrounding the vascular arborization, the tendon, and the head of the femur there was unavoidably a small circulation. It can introduce, however, no significant error to consider that the preparation described is composed of muscle alone.

In the course of an experiment every precaution was taken against cooling, drying, occluding the blood supply, or stimulating the nerve of the preparation. Large gauze sponges moistened with warm salt solution prevented drying of the tissues and aided in keeping the muscles warm during the three to four hours required for the experiment. The muscle was carefully established on a layer of moist gauze in an electrically warmed box equipped with a thermometer. In addition, the rectal temperature of the animal, which was by the decerebration deprived of the power of thermal regulation, was kept as nearly constant as possible on the electrically warmed operating table.

c. Collection of the blood. In the experiments on trained animals the

samples of blood were collected in oiled syringes from the femoral vessels, the arterial sample usually immediately following the venous. When the latter was being obtained care was taken to prevent a reflux of blood from the abdominal veins, and as far as possible to avoid any obstruction to the flow of blood through the muscle.

In the experiments on the decerebrate animals, and in those on the isolated muscles in which it was desired to determine the rate of blood

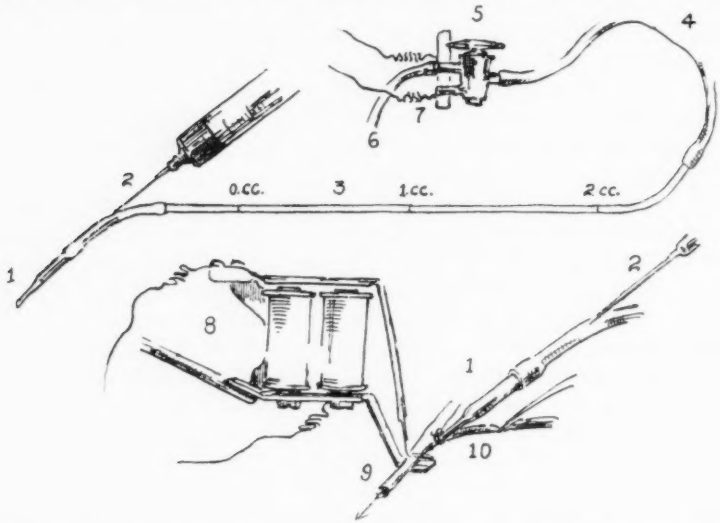

Fig. 2. Diagram of blood flow measuring device. *1*, glass cannula with beveled expanded tip; *2*, needle for injecting heparin through wall of rubber tube; *3*, horizontal calibrated measuring tube; *4*, rubber tube connecting measuring tube with two-way stopcock; *5*, two-way stopcock with electric contact for magnetic occluder; *6*, rubber tube from air pressure of 30 to 40 cm. of water; *7*, to room air; when stopcock is turned to this side for connection with room air magnetic occluder is activated; *8*, magnetic occluder for closing femoral vein; *9*, femoral vein; arrow points in direction of blood flow; *10*, branch of femoral vein with cannula inserted in its junction with femoral.

flow for computation of the oxygen consumption, the following technique was employed. The largest side branch of the femoral vein was selected as the one for the cannula, and the peripheral branches not issuing from the isolated muscles were tied off. Under the selected branch two fine silk ligature threads were passed, one of which was used to tie off the vein at a distance of 1 to 2 cm. from its intersection with the main vein. A cannula (see fig. 2, *1*) having a bevelled orifice with a small expanded

lip and fitted with a short piece of pure rubber tubing, was found to work best. The cannula and tube were completely filled with a solution of heparin and the end of the tube clamped to retain the liquid. The main vein, *9*, was clamped off gently above and below the side branch, and as expeditiously as possible, through a nick in the wall of the branch close to the bifurcation, the cannula, *10*, was inserted. Its tip was gently pushed past the valve into the lumen of the main vein and the ligature tied to hold it in place. Then, usually less than two minutes later, the clips on the main vein were removed and the normal circulation resumed. Finally, the cannula was gently manipulated until the bevel was parallel to the wall of the main vein and just within it, with the ligature holding on the lip of the glass at its very extremity. In that position it was firmly held by a clamp so that the flow of blood was unobstructed in the main vein.

To the end of the rubber tube on the cannula was connected a horizontal, straight, uniform glass tube, *3*, of about 2 to 3 mm. bore calibrated in cubic centimeters—the first mark being 10 cm. distant from the end connected to the cannula. The far end of the glass tube was connected by a rubber tube to a two-way glass stopcock, *5*, by means of which the calibrated tube could be connected at will either with the atmospheric pressure or with a source of air pressure of a few millimeters of mercury. Through the side of the short rubber tube connecting the venous cannula to the calibrated tube penetrated the needle, *2*, of a syringe filled with the heparin solution.

A device operated by an electromagnet, *8*, was adjusted to the femoral vein central to the side branch, so that it was possible instantly to occlude the vein or to leave it unobstructed. By obvious electrical connections with the two-way stopcock, *5*, whenever the valve put the measuring tube in connection with the room air, the vein occluder closed the femoral vein and diverted the blood via the branch into the cannula; and when the cock was in the intermediate position, or connected with the air pressure, the occluder left the vein open. Thus, it was possible to divert the flow of blood at will from the main vein and allow it to advance along the measuring tube, *3*, and then to return it by the positive pressure to the vein. Coagulation of the blood in the measuring tubes was prevented by rinsing out the cannula with a small injection of the heparin solution after each measurement.

In making accurate measurements of flow, there are a few refinements which it is necessary to observe. First, if the cannula is properly inserted there will be no damming of blood behind it in the peripheral part of the femoral vein; and there will be a rhythmic reciprocating motion of the meniscus in the calibrated tube, corresponding to respiratory fluctuations in venous pressure in the animal's abdomen and indicating a free communication through the system. Again, if the flow is to be measured

accurately without distorting the circulation in the muscle, there must be if possible no difference in the pressure on the venous blood whether it is passing in its normal channel or into the measuring tube. Thus, when setting up the measuring tube, it was always connected first with the central portion of the femoral vein and elevated to the height to which the abdominal venous pressure would just raise the blood. At that level it was then firmly held in a horizontal position. Finally, as a solution of heparin in physiological salt had been used to rinse out the cannula and so prevent clotting, by its difference in viscosity from that of the blood, a factor capable of varying the rate of flow in the measuring tube was introduced. Accordingly, the blood was allowed to flow out and back three times in the calibrated tube, in order to get rid of any excess of heparin solution and to wet the tube walls thoroughly before taking a measurement or a sample of blood.

The taking of the sample was easily accomplished as follows: The needle of a carefully oiled, 20 cc. syringe was passed through the wall of the rubber tube on the venous cannula until the end of the needle was very near the constricted tip within. Then, after sending the blood down and back along the calibrated tube three times, the blood was again diverted down the tube until, just as the advancing meniscus reached the last mark, the piston of the syringe was steadily withdrawn at such a rate as to keep the meniscus at that mark. This, of course, meant that the blood was taken into the syringe only so fast as it entered the calibrated tube. It was thus impossible for changes in the rate of blood flow in the muscle to be produced by venous stasis. As soon as the syringe was filled and the needle withdrawn a small injection of heparin was made into the cannula to prevent clotting.

The arterial samples were taken from a small cannula in a branch of the opposite femoral artery, after washing out the heparin solution in the cannula and its rubber tube with a little blood allowed to escape slowly before the blood for the sample itself was taken. Only in the last two experiments were the arterial and venous samples taken simultaneously, because it was feared that the removal of the arterial sample might produce a vasoconstriction affecting the limb or muscle under observation which could possibly cause fluctuations in the metabolism and gas exchanges of the tissues. Consequently, in all experiments on the whole leg where a simultaneous removal of arterial blood would have meant a rapid withdrawal, the arterial sample was taken immediately after the venous. It seemed reasonable to suppose that the venous return from the isolated limb, certainly from the muscle, would be so small in comparison with the total volume of blood returning to the heart that its loss could hardly produce significant changes in the blood flow of the pul-

monary circuit and hence in the gaseous content of the arterial blood following. In the last two experiments on isolated muscle, exactly simultaneous samples of blood were removed, because the venous flow was so slow that the corresponding loss of blood from the general arterial side could certainly produce no vasoconstriction.

The 20 cc. samples of blood were at once expelled without contact with air into glass containers where they were stored over mercury. Coagulation and glycolysis were prevented by thorough mixing of the blood with enough potassium oxalate and sodium fluoride, previously dusted in the containers, to make 0.2 per cent and 0.1 per cent solutions respectively. The oxalate and fluoride had been previously corrected to a pH of 7.3. In addition, the blood samples were placed in an ice box and kept there except when it was necessary to remove samples for analysis.

d. Analysis of the blood. In addition to O_2 and CO_2 contents, it was desired to have the oxygen capacity of the blood and a point in the oxygenated carbon dioxide dissociation curve approximately at alveolar carbon dioxide tension. Both were determined at the same time on the same sample. Shortly after the collection, 10 cc. of each sample of blood was forced into a tonometer containing a mixture of O_2 and CO_2 which, at a temperature of 38°C. and a barometric pressure of 760 mm. of mercury, had a CO_2 tension of 38 mm. and an O_2 tension of almost 700 mm. of mercury. The gas mixture was kept under high pressure in a steel cylinder. It was found convenient to use this mixture because not only were the CO_2 and O_2 tensions constant and determined from a single analysis of the contents of the cylinder, but at the high partial pressure of O_2 employed, the O_2 capacity of the blood was found to have a constant and presumably maximal value.

The tonometer was rotated for a few minutes in a water bath at thirty-eight degrees. Then the pressure of the gas mixture, which had been raised by the increased temperature, was reduced to atmospheric by allowing the extra gas to escape under water and the equilibration continued for fifteen minutes. The tonometer was removed from the water bath and the same gas mixture was passed through it for about one minute before it was again returned to the water bath and the above procedure repeated.[2] The blood which had thus been twice saturated was collected over mercury in other containers and placed in the ice box.

One cubic centimeter samples of the arterial and venous bloods as drawn and also after being equilibrated with the $O_2 + CO_2$ mixture, were analyzed for O_2 and CO_2 by the method of Van Slyke and Neill (10). When duplicate determinations yielded a larger difference than 0.3 vol-

[2] It is realized that this second washing of the tonometer with gas introduced the possibility of changing the volume by evaporation.

ume per cent, further analyses were performed. Thus O_2 and CO_2 contents, CO_2 capacity at 38 mm., and O_2 capacity were estimated.

e. Collection and analysis of the expired air. Except for the trained dogs, which wore masks, expired air was collected in a Tissot spirometer through a tracheotomy tube and two Tissot valves before, during and after blood

TABLE 1a

Blood gas analyses in experiments on the whole leg

Figures are volumes per cent

1	2	3	4	5	6	7	8	9	10	11
	CONTENTS						CAPACITIES			
EXPERIMENT NUMBER	Carbon dioxide			Oxygen			Carbon dioxide		Oxygen	
	Arterial	Venous	Venous corrected	Arteria	Venous	Venous corrected	Arterial	Venous	Arterial	Venous
1	30.30	36.42	36.42	24.61	19.06	19.06	37.86	36.98	28.49	28.45
	24.93	36.22	36.22	24.97	14.65	14.65	36.51	35.94	27.88	28.02
2	32.38	38.69	38.75	24.95	17.65	17.53	35.90	35.83	28.86	29.07
	34.84	39.90	40.31	26.65	17.21	16.39	37.48	37.45	27.50	28.79
4	34.53	39.06	39.06	23.01	14.13	14.13	38.94	36.64	26.16	26.26
	30.36	41.04	41.04	23.14	11.66	11.66	36.44	35.49	26.34	26.41
5	24.88	26.64	26.64	26.29	24.21	24.21	30.40	30.36	29.28	29.40
	25.73	27.16	27.16	25.77	23.21	23.21	31.72	30.98	28.53	28.52
6	31.50	34.82	34.82	22.54	17.98	17.98	34.60	33.47	25.89	25.95
	31.55	34.92	34.92	22.54	17.11	17.11	34.27	33.50	25.42	25.34
19	27.06	29.44	29.61	19.71	16.28	15.83	35.45	34.40	22.07	22.63
	25.59	28.25	28.34	20.01	17.78	17.53	35.04	33.88	24.23	24.57
3	37.35	40.34	40.48	16.10	12.18	11.98	39.53	39.33	18.48	18.76
7	32.77	36.26	36.38	16.49	12.59	12.38	34.39	34.16	19.17	19.46
10	38.80	42.02	42.31	23.30	17.39	16.82	39.35	38.18	26.61	27.46
11	37.10	40.17	40.28	24.69	20.03	19.77	38.05	38.21	27.65	28.00
15	32.70	33.78	33.83	26.17	24.95	24.77	35.97	35.91	29.74	29.94
16	26.25	40.24	40.07	22.75	2.50	2.55	32.48	29.54	27.99	27.38

samples were taken. Usually the samples of expired air taken during the collection were analyzed for O_2 and CO_2 by the Haldane apparatus to determine the respiratory quotient of the animal at the time blood was being drawn. The respiratory quotient was not corrected for protein metabolism. This was not essential since the special purpose of the

analysis of expired air was to compare the gaseous ratios of the whole animal with that of the blood of the femoral vessels. In any case the error in the respiratory quotient could not be large.

TABLE 1b

Blood gas analyses in experiments on isolated muscles

Figures are volumes per cent

1	2	3	4	5	6	7	8	9	10	11
	CONTENTS						CAPACITIES			
EXPERIMENT NUMBER	Carbon dioxide			Oxygen			Carbon dioxide		Oxygen	
	Arterial	Venous	Venous corrected	Arterial	Venous	Venous corrected	Arterial	Venous	Arterial	Venous
8	27.90	39.23	39.31	22.15	7.41	7.34	32.39	30.17	24.64	24.87
9	26.61	34.60	34.74	17.42	8.52	8.35	32.14	31.75	20.74	21.11
	30.07	34.77	34.77	13.41	6.40	6.40	31.93	31.66	20.25	20.33
12	26.75	33.72	33.67	18.95	9.12	9.19	32.05	29.69	21.99	21.82
13	24.05	28.95	28.95	19.38	11.64	11.64	29.79	28.14	21.79	21.86
14	30.21	35.91	36.11	18.73	11.66	11.34	32.55	31.53	21.36	21.90
	25.19	28.95	28.95	18.86	12.84	12.84	32.27	30.82	31.35	20.25
17	26.45	29.82	29.91	20.15	15.03	14.82	37.16	36.28	22.89	23.19
	27.32	29.60	29.84	22.24	16.70	16.05	36.91	34.62	24.93	25.87
18	29.15	30.85	30.71	23.01	20.04	20.57	35.64	33.58	27.00	26.16
	22.09	26.23	26.23	25.75	22.00	22.00	31.60	32.98	29.21	28.66
21	21.21	29.59	29.34	24.96	19.71	20.53	30.90	29.11	29.90	28.78
	27.67	29.95	29.87	22.45	16.42	16.63	32.02	29.91	27.79	27.45
20	9.74	16.66	16.76	19.89	12.97	12.67	21.99	22.14	21.38	21.97
	9.87	17.23	17.37	21.51	13.60	13.54	21.74	21.56	24.63	24.72
22	26.04	30.49		21.32	12.70					
	26.15	31.87		21.46	11.51					
	26.55	32.50		24.79	13.66					
	26.49	31.58		24.19	14.64					

EXPERIMENTAL RESULTS. *a. Blood gas analyses.* Tables 1a and 1b contain all the data obtained on the blood. The first eighteen determinations were made on the whole leg, the following nineteen on isolated muscles. Simultaneous arterial and venous O_2 and CO_2 contents, venous

figures corrected for changes in concentration of the blood, as well as the respective O_2 and CO_2 capacities may be read across the lines. The average differences in the whole leg in artery and vein for the CO_2 and O_2 contents are 4.97 and 6.77 volumes per cent respectively, while for the isolated muscle they are 5.12 and 6.63 volumes per cent. From columns eight and nine it is seen that usually there is a fall in the CO_2 dissociation curve as the blood passes through the tissues of the legs. The changes in the CO_2 dissociation curves of succeeding samples of arterial blood are insignificant in comparison, especially when we remember that the change in venous blood is produced by a single passage of the blood, while the length of time between arterial samples is much longer. The last two columns of the tables contain the O_2 capacity data. In all but nine of the thirty-three observations there was a rise of the O_2 capacity of the venous blood averaging 0.35 volume per cent and reaching in one case a maximum of 1.29 volumes per cent. This indicates that concentration of the venous blood is the common condition, and indeed in five of the nine cases in which dilution was observed the fall in the O_2 capacity was less than 0.2 volume per cent, a figure within the error of the method.

b. Concentration of the blood. Barcroft and Kato (11) tried, by quantitating the hemoglobin content of arterial and venous blood, to determine whether in passing through the isolated gastrocnemius muscle of the dog the blood became concentrated. Their conclusion was that at rest only a slight concentration occurred. According to the experiments of Meakins, Dautrebande and Fetter (12), blood, especially under stasis, gives up fluid as it passes through the tissues of the forearm. Under such circumstances the hemoglobin-containing portion of the blood would be increased either by a swelling of the cells or a loss of plasma, both of which probably occur. In our experiments on non-isolated muscle some minor damage may have been done to the lymphatic vessels in isolating the femoral vein, but in the completely isolated muscles most of the lymphatic channels were probably destroyed. In the latter, perhaps from this cause, some edema appeared to develop, although in these experiments unfortunately no comparison by weight was made with the opposite undisturbed muscle. For the non-isolated muscles there are three venous O_2 capacities below and fifteen above the corresponding arterial capacity. For the isolated muscles nine show an increased O_2 capacity of the venous blood, and six show a decrease. Judging from the non-isolated muscles where, owing to the slight damage to lymphatics, the conditions are probably more nearly normal, there is usually a slight definite increase of the hemoglobin carried in a given volume of venous blood. The return to the previous lower O_2 capacity of the arterial blood may then be accounted for in part by the readdition of lymph or the change in pH of the blood in its arterialization in the lung.

From tables 1a and 1b it is evident that a fall of the CO_2 dissociation curve of the blood in passing through the tissues takes place in all but three of the experiments. It is also to be seen here, that in the twelve experiments in which two arterial CO_2 capacities were taken, there is only a slight tendency to variation. Excluding one long interval of two hundred and ten minutes, the average time elapsing between the first and second arterial samples was thirty-three minutes. In three experiments there was a rise of the second curve averaging 1.04 volumes per cent. In the nine others the capacity for CO_2 of the arterial blood shows a fall averaging 1.07 volumes per cent. In attempting to explain the progressive fall of the CO_2 curve of the arterial blood we have the similar results of the Medical Research Committee (13) who showed a fall of the alkali reserve of decerebrate cats accompanying the failing circulation. Macleod (14) has shown that in anoxemia lactic acid may appear in the blood stream in considerable quantity. These facts seem to offer a reasonable explanation of the cause of the downward course of the arterial CO_2 curve. If, furthermore, we assume the source of this acid to be the tissues, there is at hand a convenient explanation for the lower level of the CO_2 curve of the venous sample relative to that of the arterial observed in most of these experiments. On the other hand, the cause of this last effect and possibly of the progressive fall of the arterial curve may be ascribed to a transfer of alkali from the blood to the tissues. The stasis experiments of Dautrebande, Davies and Meakins (15) and of Peters and his associates (16) demonstrate a transfer of base to the tissues instead of an accumulation of acid in the blood. These results are unexpected because it seems probable that long continued anoxemia of the forearm, largely composed of muscle, should produce some lactic acid, which Barr and Himwich (17) have shown in exercise may pass from the tissues to the blood stream and back again. On the contrary, Haldane and Quastel (18) claim that muscle cell membranes are not very permeable to lactic acid. Lactic acid has, however, been shown by Jannsen and Jost (19) to be present in greater amount in the venous than in the arterial blood of resting muscles. To explain the progressive fall of the arterial curves it would also be necessary to assume that the alkali lost from the blood is either lodged in the tissues or excreted by the kidneys. It seems more probable that some fixed acid such as lactic acid is produced in the tissues by the abnormal conditions of decerebration and poured into the blood stream; but it may be that the tissues take base from the blood, or that the lowering of the curve is due to both mechanisms combined, or even to equilibria between other substances. Into this obscurity our experiments, presenting only analyses of CO_2 and O_2 without determinations of the relations of other ions, can throw no light; they merely increase the existing evidence

for a definite fall of the CO_2 dissociation curve of the blood in passing unobstructedly through a resting tissue.

c. Oxygen consumption of muscle. Table 2 summarizes the data on the O_2 consumption of twelve determinations in five decerebrate fasting animals with resting isolated muscles of known weight. It is possible that the decerebrate rigidity may have increased the figures slightly. The average figure, 0.36 cc. of O_2 per gram per hour, agrees with the previous data obtained on gastrocnemius muscle fairly well. Verzár (20) in anesthetized, and Langley and Itagaki (21) in decerebrate cats, ob-

TABLE 2

Simultaneous oxygen consumption of isolated muscles and of entire animal

EXPERIMENT NUMBER	WEIGHT OF ANIMAL	TEMPERATURE OF MUSCLE	WEIGHT OF MUSCLE	BLOOD FLOW PER HOUR PER GRAM	O_2 UTILIZED FROM 100 CC. BLOOD	O_2 CONSUMPTION PER HOUR PER GRAM MUSCLE	O_2 CONSUMPTION PER HOUR PER GRAM ENTIRE ANIMAL
	kgm.	*°C.*	*grams*	*cc.*	*cc.*	*cc.*	*cc.*
17	23.31	39.4	110	4.08	5.33	0.217	0.508
	23.31	40.3	110	3.72	6.19	0.230	0.568
18	16.12	38.0	72	9.42	2.44	0.230	0.447
	16.12	38.0	72	6.42	3.75	0.241	0.461
20	14.57	41.7	64	7.98	7.22	0.576	0.678
	14.57	37.0	64	6.84	7.96	0.544	0.636
21	29.7	37.5	90	5.34	4.43	0.236	0.404
	29.7	38.3	90	4.62	4.79	0.221	0.405
22	26.8	39.6	112	5.34	8.62	0.460	0.524
	26.8	37.5	112	4.74	9.95	0.472	0.510
	26.8	37.5	112	4.02	11.13	0.448	0.506
	26.8	37.8	112	4.20	9.55	0.401	0.496
Averages.....						0.356	0.512

served slightly smaller values averaging 0.27 cc. per gram per hour. The O_2 usage in this muscle in anesthetized dogs was found by Barcroft and Kato (11) to be somewhat greater, 0.64 cc., a figure greater than our largest. Chauveau and Kaufmann (22) in the masseter and levator muscle of the upper lip of the horse, perhaps not under perfect resting conditions but certainly not affected by any form of anesthetic, found an O_2 consumption of 0.34 cc. per gram per hour. These figures are smaller than those obtained by the Warburg method of studying thin slices of tissue. Meyerhof and Himwich (2) found an O_2 consumption by the latter method

of 1.1 cc. per hour in the diaphragm of the full grown rat, and 2.2 cc. for the mouse.

The present results are of special interest because they are accompanied by those of the simultaneous oxygen consumption of the entire animal. Since muscle is thought to have so large a part in the production of the basal metabolism, it is surprising to find that in every case the oxygen consumed per gram of muscle is less than that per gram of entire animal. The average oxygen intake per gram of muscle per hour is 0.356 cc. or two-thirds of 0.512, the average per gram per hour for the entire body. Assuming that the muscles weighed 8.82 kilos or four-tenths of the average weight of the entire dog, 22.1 kilos, they utilize 3140 cc. of oxygen per hour, an amount less than three-tenths of the mean oxygen consumption of the body of 11,315 cc. per hour. Apparently organs other than resting muscles are larger consumers of the oxygen taken in by the body under resting conditions. This is in agreement with the work of Hunt and Bright (23), whose results show a caloric production of 0.5 to 1.0 calorie per kilogram per hour for muscle in contrast to an average figure for the viscera of 2 to 3 and for the liver of 10 to 20 calories per kilogram per hour.

d. Method of calculating the respiratory quotient from the blood samples. As already mentioned, respiratory quotients on the whole leg of the dog have been determined by Doisy and Beckmann (6) from analyses of appropriately taken arterial and venous samples. The respiratory quotient was calculated by dividing the increase in CO_2 by the decrease in O_2. Such a procedure is valid under two conditions: first, that the concentration of the blood does not change in its passage through the tissues; and, second, that the CO_2 dissociation curve of the arterial blood is unaltered. Both criteria will be considered in turn: Barcroft (11) pointed out the fact that to determine the O_2 consumption of a tissue it is necessary to know the difference in concentration of red blood corpuscles in arterial and venous blood, as the oxygen carriers; and showed that there was only a slight increase in concentration of blood passing through resting muscle. Similarly, in many of the present experiments there was a slight concentration of the venous blood (table 1b). For this change in concentration a correction was applied as in the following example: Given O_2 capacities of 20 volumes per cent and 21 volumes per cent in arterial and venous blood respectively, and O_2 contents of 19 and 14 volumes per cent, then $14 \times \frac{20}{21} = 13.3$ would be the corrected venous content, i.e., venous O_2 content $\times \frac{\text{art. } O_2 \text{ cap.}}{\text{ven. } O_2 \text{ cap.}}$, and the oxygen utilization would be 5.7 volumes per cent instead of 5.0 volumes per cent. When there is no change in capacities, the correction becomes unity and the observed content is correct.

With a change in concentration the venous CO_2 content also calls for a correction. The basis of the correction again lies in the O_2 capacity determination. The blood corpuscles form about (*a*) $\frac{45}{100}$ of a given volume of blood. The increase in the O_2 capacity of the venous blood indicates that the relative volume occupied by the corpuscles became larger at the expense of the complementary volume of plasma in a given amount of blood. This increase in O_2 capacity is expressed mathematically as (*b*) $\frac{\text{ven. } O_2 \text{ cap.} - \text{art. } O_2 \text{ cap.}}{\text{art. } O_2 \text{ cap.}}$ and being directly proportional to the change in volume of the corpuscles or plasma may be expressed here in absolute units as (a) multiplied by (b) or $\frac{45}{100} \times \frac{\text{ven. } O_2 \text{ cap.} - \text{art. } O_2 \text{ cap.}}{\text{art. } O_2 \text{ cap.}}$

The ratio of CO_2 carried in equal volumes of plasma, whole blood, and corpuscles is 6 : 5 : 4. Hence the displaced plasma holds (*c*) six-fifths the amount of CO_2 of an equal quantity of whole blood, and a given volume of corpuscles contains two-thirds the amount of CO_2 of the plasma it displaces. Since two-thirds of the volume of CO_2 lost with the plasma is carried in the corpuscles, in replacing corpuscles with plasma it is necessary to correct only for (*d*) one-third the amount of CO_2 carried by the plasma. Combining (*a*), (*b*), (*c*), and (*d*), the correction becomes

$$\text{ven. } CO_2 \text{ content} \times \frac{\text{ven. } O_2 \text{ cap.} - \text{art. } O_2 \text{ cap.}}{\text{art. } O_2 \text{ cap.}} \times \frac{45}{100} \times \frac{6}{5} \times \frac{1}{3}.$$

with sufficient accuracy the corrected venous CO_2 content =

$$\text{venous } CO_2 \text{ content} + \frac{\text{ven. } O_2 \text{ cap.} - \text{art. } O_2 \text{ cap.}}{\text{art. } O_2 \text{ cap.}} \times \frac{1}{5} \times \text{ven. } CO_2 \text{ content},$$

which may be written:

$$\text{ven. } CO_2 \text{ content} \left(1 + \frac{\text{ven. } O_2 \text{ cap.} - \text{art. } O_2 \text{ cap.}}{\text{art. } O_2 \text{ cap.}} \times \frac{1}{5}\right).$$

If there is no change in concentration, the second term in the parenthesis becomes zero. When the venous blood dilutes, the sign of the second term is minus.

A second condition necessary for consideration is the height of the CO_2 dissociation curve. Murlin, Edelmann and Kramer (24) pointed out the fact that when extra CO_2 is eliminated from the lungs with a rise in the apparent respiratory quotient of the whole animal, the CO_2 content of arterial blood falls. Peters (25) showed that such variations in the CO_2 content are associated with corresponding shifts in the CO_2 dissociation curve. The progressive fall of the arterial CO_2 curve is in these experiments, as has been shown above, the more common condition. (See tables

1a and 1b.) This is probably caused by the decerebration (13) and consequent gradual failure of the circulation resulting in the production of acid in the body tissues from anoxemia. However, the normal level of the CO_2 curves for the blood of the dog, and their very slow fall attest that the condition of the circulation of these animals at the time of the taking of the blood samples was excellent and undergoing very slow deterioration. In the absence of definite knowledge of the exact changes taking place in the tissues or of the consequence of such changes on the action of the respiratory center and thus on the constancy of the alveolar CO_2 tension, it is difficult to do more than guess at the magnitude of the effect of the process on the respiratory quotient of the expired air. The outside limits of such changes may be surmised by assuming that the process is mediated by a uniform progressive accumulation of acid in all the tissues of the body including the blood; the calculations presuming in addition a constant alveolar CO_2 tension.

On this basis, then, an average fall of 1.07 volumes per cent between successive arterial CO_2 curves, in thirty-three minutes from two liters of blood, a fair estimate of the blood volume in a twenty kilogram dog, would have given off 2.14 cc. of CO_2. With a proportionate loss of CO_2 from the remaining six-tenths of the body which is water, 128.4 cc. of CO_2 would have been evolved totalling, with the contribution of the blood, 149.8 cc. in thirty-three minutes. Data that we obtained on the O_2 consumption of such a dog suggest a total production of CO_2 from oxidation of about 150 cc. per minute, which amounts to 4950 cc. of CO_2 in thirty-three minutes. The effect of an increased production of 149.8 cc. of CO_2 from such an animal in thirty-three minutes on a respiratory quotient in the vicinity of 0.75 would be to raise it 0.023, a result less than half the mean deviation of the average of our figures for the respiratory quotients of the expired air. Also this figure is practically 0.024, the magnitude of the average difference between the determinations of the external respiratory quotients in a single experiment excluding experiment 14.

The figures for the corresponding venous CO_2 dissociation curve, as was to be expected if the tissue under investigation was representative of general conditions in the body, show a fall in eight of the nine cases in which the arterial CO_2 curve was lower, and practically no rise (0.03 volume per cent) in the ninth. In the three experiments in which the second arterial blood showed the higher CO_2 capacity, the corresponding venous blood was altered in the same way, averaging a rise of 1.01 volumes per cent compared to 1.04, the average rise of the three arterial bloods. The average fall of the nine second arterial curves below the first was 1.07 volumes per cent; for the venous blood this average was 0.79 volume per cent. Thus it seems probable, both from the absence of abnormal quotients and

from the data just discussed that the external respiratory quotient of the animal was not significantly affected by the progressive rising or falling of the CO_2 dissociation curve in successive samples of arterial or venous blood.

So far, however, these considerations have not included the effect of the lowering of the curve on the respiratory quotient of the tissues. We have shown that in the majority of the experiments the second CO_2 dissociation curves of both arterial and venous blood are lower. If this means a production of acid in the body produced by the decerebration and operation, the chief source of the acid must be the tissues. The lowering of the curve in the blood is, then, principally the reflection of an acidity in the tissues which may well have similarly affected their "dissociation curve." By this means a neutralization of bicarbonate in the tissues with a consequent rise in the CO_2 tension may well have accompanied the fall of the dissociation curve of the blood. A certain amount of extra CO_2 would in this way be given up to the circulating blood stream with an apparent rise in the respiratory quotient of the tissue itself. This rise in the tissue respiratory quotient would then be accompanied by a change in the same direction, perhaps of a similar magnitude, in the external respiratory quotient, both from the primary changes in the tissues and the reflection of that process in the fall of the CO_2 dissociation curve of the blood. It may reasonably be assumed that the general trend of both respiratory quotients in the quiescent animals is identical, for otherwise it would be necessary to have either a storage or else an elimination of quantities of "extra" CO_2 to cause a difference. Leaving aside this rather improbable hypothesis we may infer that the effect of the usual moderate lowering of the arterial CO_2 curve would be within approximately the same limits for tissue as for the expired air. It is possible, then, that no significant difference in the two respiratory quotients is produced in these experiments by the progressive fall of the arterial CO_2 curves, especially as the change in the level of the curve of the blood is at most slow.

On the other hand, it is possible that differences in the level of the dissociation curve of the arterial and venous bloods may produce significant alterations in the apparent respiratory quotient of the tissues. Therein the exchanges with the blood are not confined to CO_2 and O_2; and the problem, in these experiments, in which no data on the relations of other ions exist, seems too ill-defined even for rough calculations. However, consideration of the fact that in a muscle subjected to the unavoidable trauma of isolation there might result conditions not truly representative of the body as a whole, urged us strongly to attempt to find a correction for this factor. That such a condition may exist in certain experiments is undeniably suggested by the last experiment on an isolated muscle, 22. In that case, within a period of an hour, four determinations of

the respiratory quotient of expired air fell close together at a normal level, but about 0.2 higher than four practically identical determinations made on the blood from the isolated muscle. It is to be regretted that CO_2 capacities were not available on this animal's blood. Accordingly, in the other experiments, corrections for this factor were attempted, but found to produce no lessening of the discrepancy between blood respira-

TABLE 3a

Simultaneous respiratory quotients of whole leg and of entire animal

1	2	3	4	5	6
EXPERIMENT NUMBER	TYPE OF EXPERIMENT	R.Q. OF LEG FROM A-V SAMPLES	R.Q. OF LEG USING CORRECTED VENOUS SAMPLES	R.Q. OF ANIMAL FROM EXPIRED AIR	DIFFERENCE BETWEEN 4 AND 5
1	Decerebrate, fasted	0.91	0.91		
		1.09	1.09		
2	Decerebrate, fasted	0.86	0.89		
		0.54	0.53		
4	Decerebrate, fasted	0.51	0.51	0.81	−0.30
		0.93	0.93	0.82	+0.11
5	Decerebrate, fasted	0.85	0.85	0.78	+0.07
		0.56	0.56	0.76	−0.20
6	Decerebrate, fasted	0.73	0.73	0.77	−0.04
		0.62	0.62	0.74	−0.12
19	Decerebrate, fasted	0.69	0.89	0.68	+0.21
		1.19	1.11	0.73	+0.38
3	Fasted, trained	0.76	0.76		
7	Fed, trained	0.90	0.88	0.88	0
10	Fasted, trained	0.55	0.54	0.75	−0.21
11	Fasted, trained	0.66	0.65	0.78	−0.13
15	Depancreat., trained	0.89	0.81		
16	Depancreat., trained	0.69	0.68		
Averages.....		0.77 ±0.09	0.77 ±0.08	0.77 ±0.03	

tory quotients and those of the expired air in individual cases. In the face of these difficulties there appeared the fact that the average of all the determinations on the blood comes close to the average of the respiratory quotient on the whole animal. It may be supposed that whatever factors might cause the individual discrepancies would tend to balance in the average of the thirty-seven determinations.

e. Respiratory quotients. Tables 3a and 3b contain all of the respiratory quotients obtained in all the experiments on the whole leg and on the isolated muscle respectively. In column three of each table are tabulated the respiratory quotients calculated from the directly observed differences

TABLE 3b

Simultaneous respiratory quotients of isolated muscle and of entire animal

1	2	3	4	5	6
EXPERIMENT NUMBER	TYPE OF EXPERIMENT	R.Q. OF LEG FROM A-V SAMPLES	R.Q. OF LEG USING CORRECTED VENOUS SAMPLES	R.Q. OF ANIMAL FROM EXPIRED AIR	DIFFERENCE BETWEEN 4 AND 5
8	Decerebrate, fasted	0.77	0.77	0.86	−0.09
9	Decerebrate, fasted	0.90	0.90	0.73	+0.17
		0.67	0.67	0.73	−0.06
12	Decerebrate, fasted	0.71	0.71	0.79	−0.08
13	Decerebrate, fasted	0.63	0.63	0.85	−0.22
14	Decerebrate, fasted	0.81	0.80	1.00	−0.20
		0.63	0.63	0.82	−0.19
17	Decerebrate, fasted	0.66	0.65	0.68	−0.03
		0.41	0.41	0.70	−0.29
18	Decerebrate, fasted	0.57	0.64	0.78	−0.14
		1.11	1.11	0.75	+0.36
21	Decerebrate, fasted	0.64	0.70	0.81	−0.11
		0.38	0.38	0.80	−0.42
20	Decerebrate,	1.00	0.70	0.83	−0.13
	Depancreatized	0.93	0.94	0.86	+0.08
22	Decerebrate, fasted	0.52		0.76	
		0.58		0.77	
		0.53		0.77	
		0.53		0.78	
Averages.....		0.68 ±0.09	0.71 ±0.10	0.80 ±0.04	

in O_2 and CO_2 content of arterial and venous samples, while in column four are listed the respiratory quotients, calculated from the values for venous O_2 and CO_2 corrected for concentration of the blood in passing through the tissues. It is obvious that the differences between the respiratory

quotients of the blood and of the expired air given in column six are large. Even duplicate determinations of the respiratory quotient from the blood, made in a single experiment in which, as indicated by constancy of temperature and external respiratory quotient, conditions should be as nearly uniform as possible, show large differences both between each other and in comparison with the expired air. It therefore becomes necessary to treat the data statistically.

The respiratory quotients of the expired air in non-isolated and isolated muscle experiments vary respectively between 0.68 and 0.82, and 0.68 and 1.00, a quite normal range, with a mean deviation for each series of ±0.02. In contrast to these, the corrected respiratory quotients determined from the blood vary for the two series between the limits of 0.51 and 1.11, and 0.41 and 1.11. The averages of the respiratory quotients with double their mean deviation as determined from the expired air of dogs with non-isolated and isolated muscles are 0.77 ± 0.03 and 0.80 ± 0.04 respectively. The corresponding averages of the corrected respiratory quotients from blood are 0.77 ± 0.08 and 0.71 ± 0.10.

In analyzing samples of blood, in most cases more than two analyses were made if the difference between duplicates was found to be greater than 0.3 volume per cent. Any result differing by more than 0.5 volume per cent from any one of two or more others was discarded. Such divergent determinations were not uncommon. It is difficult in the absence of a large series of analyses on a single sample, and with our inability to secure uniformly good results, to estimate the limits of error of the final figure taken for the gas content of a sample. Probably the maximum variation is as great as ±0.3 volume per cent. The greatest error in taking the difference between arterial and venous gas contents is thus ±0.6 volume per cent. The average difference in the CO_2 content of arterial and venous blood was approximately 5.0 volumes per cent; for O_2 it was 6.7 volumes per cent. Consequently, from errors in blood gas analysis alone, exclusive of other experimental variations, for CO_2 and O_2 differences of 5.0 ± 0.6 and 6.7 ± 0.6 volumes per cent respectively, the limits of error in the determination of the respiratory quotient would be ± 0.16. This figure would be increased in those cases in which the differences in gaseous content of arterial and venous samples were less than the average figures utilized above. Probably a mean deviation in the respiratory quotient of ±0.13 may be expected from this source alone.

In addition to deviations produced by the analyses of the blood and variations in the experimental technique, it is possible that the divergences have another cause. The respiratory quotient based on the expired air represents an average of the metabolic activities of the whole body, wherein the contribution of a particular limb or isolated muscle is submerged. However, in spite of the numerous possibilities for wide variations in the

individual respiratory quotients, it is surprising to find so few in the table—in which is included every one obtained in the investigation—at or about unity. Furthermore, the averages for the non-isolated and isolated muscle, 0.77 $\pm$ 0.08 and 0.71 $\pm$ 0.10 respectively, allow of no higher respiratory quotients than 0.85 and 0.81. These figures are close to the averages for the quotients determined from the expired air, 0.77 $\pm$ 0.03, and 0.80 $\pm$ 0.04. Thus, in contrast to former *in vitro* results, the muscle *in vivo* apparently has a respiratory quotient not of unity but more nearly that of the rest of the body.

SIGNIFICANCE OF THE RESPIRATORY QUOTIENT. Respiratory quotients of the entire leg have been determined by Doisy and Beckmann (6). Of seventeen results obtained by analyses of blood taken from the vessels of trained dogs, fourteen were found to be between 0.67 and 1.05, eight between 0.70 and 1.00. The remaining three were 0.61, 0.58 and 0.10. These determinations were not compared with the external respiratory quotient of the animal, and were not obtained from isolated muscles. However, from our experiments the difference between the quotients of the whole leg and an isolated muscle mass is seen to be small, and thus the results of these workers are in general agreement with the fact that the respiratory quotient of muscles *in situ* at rest is not unity. The reason for this apparently real difference in the behavior of excised muscles or muscles in a reduced animal (such as employed by Burn and Dale (3)) and isolated muscle in an otherwise normal animal is at present a matter for conjecture. Possibly the presence of one or more of the viscera or their secretions is necessary for normal metabolism. Certainly, as was said in the introduction, it is easier to understand the uniformity of the respiratory quotient under fixed conditions in dogs of different relative muscle mass if it is not necessary to consider that large protoplasmic mass as having a respiratory quotient fixed at unity. If muscle at rest has a quotient close to that of the whole body, no such difficulty arises.

It is well known that carbohydrate may change to fat giving a high respiratory quotient. At present the weight of evidence is against the idea that fat changes to carbohydrate with low quotients. If this be accepted, resting muscle cells, like the entire resting body, oxidize directly not only carbohydrates but also fats and proteins according to their respective availabilities.

The respiratory quotient of the expired air is determined by that of the blood. Hence, if the results of the present investigation may be applied to depancreatized dogs, their muscles must have a quotient close to 0.7. If depancreatized dogs can not burn sugar, then their muscles must be oxidizing fat directly and not after a previous conversion to carbohydrate. In fact, the theory of an obligative combustion of carbohydrate became necessary only when muscle was found to have a respiratory

quotient of unity. In the light of the present data it is no longer necessary to consider carbohydrate the sole available foodstuff for resting muscle. The above experiments do not exclude a transformation of fat to carbohydrate but they make it probable that if such change does occur it takes place in the muscles and in the body as a whole in the same relative proportions.

CONCLUSIONS

1. The respiratory quotient of mammalian muscle in the body has been studied by analyses of the O_2 and CO_2 contents and capacities of arterial and venous blood in twenty-two dogs. Thirty-seven pairs of samples were analyzed: six pairs from six trained dogs, twelve pairs from six decerebrate dogs, and nineteen pairs from ten decerebrate dogs in which the circulation through the gastrocnemius and flexor digitorum sublimis muscles had been isolated.

2. In spite of improvements in methods of preparing the animals, collecting and analyzing the blood, and calculating the respiratory quotients, there is, in individual cases, wide variation between those obtained from the blood and from the expired air. Nevertheless, few of the results of analysis of the blood are near unity or above, and the averages of both are close. The average corrected result for all isolated muscles is 0.71 ± 0.10; for non-isolated muscles 0.77 ± 0.08; and for the expired air 0.80 ± 0.04 and 0.77 ± 0.03 respectively for the two groups.

3. If, as a computation of the error suggests, it is permissible to take the average figures, the following two conclusions may be drawn. *a*. Resting muscle *in situ* has a respiratory quotient not of unity but probably close to that of the whole body. *b*. Resting muscle under normal conditions oxidizes besides carbohydrate either fat or protein or both, probably in the same proportions as does the rest of the body.

4. The average O_2 consumption per gram of muscle per hour in dogs weighing about 22 kgm. is 0.36 cc. The oxygen consumption per gram of resting muscle is only two-thirds of that calculated per gram of the entire animal.

5. The blood apparently concentrated slightly in traversing the muscle in most cases, as shown by the O_2 capacity, and was rediluted from other sources.

6. The CO_2 capacity was usually lower in the venous than in the arterial blood without, however, more than a very slight progressive fall in successive arterial CO_2 capacities.

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THE GASTRIC HUNGER MECHANISM

The Effect of Food upon the Empty Stomach and its Relation to Hunger

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When a rubber balloon is inserted into the stomach of an animal, either through the esophagus or through a gastrostomy opening, and then connected with a recording manometer, it is possible to make a tracing of the changes of intragastric pressure incident to the activity of the gastric musculature, (Boldyreff, 1904; Carlson, 1912). Carlson (1913) has described accurately the behavior of the empty stomach under these conditions.[1] He has determined that the gastric activity consists of:

1. Periods of powerful rhythmical contractions alternating with periods of relative quiescence. The period of gastric activity is designated by him as the "*hunger period.*"

2. A tonus rhythm of uniform rate but varying amplitude.

From work done on a human subject with a permanent gastric fistula, Carlson demonstrated a direct relationship between the strength of the gastric contractions and the intensity of the hunger sensation.

Pavlov (1897), through his extensive researches on dogs and man has shown that when appetite is present, the sight, smell or taste of palatable foods causes reflex secretion of gastric juice. Even associated stimuli, such as the ringing of a bell, light signals, or the entrance of the usual attendant are capable of eliciting the response after an appropriate period of training (conditioned reflexes). The suggestion is therefore obvious that stimuli which lead to so-called psychic secretion of gastric juice may at the same time cause an augmentation of the tone and the contractions of the gastric musculature (psychic tonus of Cannon (1911)).

It is the universal experience of normal persons that the sight or smell, or even the memory of palatable foods seems to induce hunger and appetite, or to intensify these sensations, if they are already present. The simplest explanation of this fact would be that the smell or taste of palat-

[1] For detailed discussion of this subject the reader is referred to Professor Carlson's monograph, 1916.

able foods initiates or augments the gastric hunger contractions, increasing the sensation of hunger by increasing the intensity of the gastric activity.

From the study of a series of observations on a man with a permanent gastric fistula, Carlson found that the facts are the very opposite to those demanded by this hypothesis. He found:

1. Inhibition of the contractions of the empty stomach by stimulation of the gustatory end-organs of the mouth;

2. Inhibition of the tone and contractions of the empty stomach by chewing indifferent substances (such as paraffin) or palatable foods;

3. Inhibition of the tone and contractions of the empty stomach by swallowing movements;

4. Inhibition of the tone and contractions of the empty stomach by alcohol, water, acids, carbon dioxide, bitters (appetizers) and a host of other substances swallowed, or placed directly into the stomach through the artificial gastrostomy opening.

THESIS. The present investigation was prompted by the almost universal experience that the sensations of hunger and appetite are augmented by the taking of food.

We have already seen that any manipulation, pleasing or otherwise, depresses the contractions of the empty stomach. How are we to account for the common observation of increased desire for food after the ingestion of small amounts of food? Can the whole syndrome be explained on the basis of increased appetite (psychic response) despite the decreased hunger (gastric inhibition)?

Furthermore, it is a common experience that the increased desire for food following the ingestion of small amounts of edible material is suppressed, 1, if the ingestion is continued to satiety; 2, if a definite period of time, usually over one-half hour, be allowed to elapse after the insufficient meal.

The experimental results herein reported are interpreted as showing that *a*, an increase in tone and activity of the stomach follows the ingestion of small amounts of food. This increases the sensation of hunger. *b*, Satiety due to sufficient ingestion, or the lapse of a sufficiently long time after the meal is due to the cessation of this increased activity, and diminution of the tone of the stomach.

EXPERIMENTAL. This investigation is based on over one hundred observations on ten adult dogs. The animals were in good health throughout the period of observation. Each animal was operated upon under ether anesthesia and with surgical asepsis, and a gastric fistula made according to the technique of Carlson (1916). Except during the operative procedure, the animals received no anesthesia or other quieting measures. They would lie quietly for hours, apparently in perfect com-

fort, and would often fall asleep. The records were made by the balloon-chloroform manometer apparatus described in a previous communication (Mulinos, 1926).

After insertion into the stomach, the balloon was fixed in place by a broad tape passing about the animal. The tube bearing the balloon is thereby anchored. This procedure was deemed advisable in view of the possibility of having the balloon carried past the pylorus into the duodenum, for the contractions of this organ resemble somewhat those under consideration in this report. Introducing the balloon into the stomach by way of a fistula offers the least possible psychic disturbance to the animal, minimizes reflex changes from mouth and esophagus, and makes possible comparable daily readings and undisturbed oral feedings with the balloon in situ.

Results. After the animal had quieted and a period of empty stomach contractions had been recorded, it was fed fifty grams of lean meat. Previous to each observation, the animals were starved for twenty-four to thirty-six hours. Consequently, they ate the proffered meat with avidity and looked for more. At first it was thought a serious drawback that the animals should show so much disturbance of their equanimity because of insufficient food. It was found, however, that they soon became accustomed to the procedure and as soon as the meat had been eaten, would fall back and lie quietly. The excessive salivation (drooling and licking chops) evident with the first few experiments, disappeared in the later ones. The writer feels certain that the results here reported are not due to any psychic disturbance. Furthermore, the gastric response to the ingested meat was the same in the first few and in the later observations. There was no evidence of the establishment of a conditioned reflex to motility, though the animals often showed evidence of expecting food when for some reason it was withheld.

After a period of empty stomach activity is recorded, the tube to the manometer is clamped, the animal is fed, and the tube is released. This procedure prevents marring of the tracing by the respiratory activity incident to the feeding.

The tracing shown in figure 1 typifies the results obtained invariably in nine of the ten dogs studied. The tenth dog gave very poor empty stomach contractions throughout the period of observations and eventually died of distemper.

a. There is an immediate cessation of the gastric hunger contractions, the drop in tone being the more marked the more active the empty stomach had been. If the animal is fed during the period of quiescence, there is no preliminary drop in tone. This suggests that the quiescent, empty stomach is not tonically contracted. The changes in pressure are not due to the presence of meat, for then the intragastric pressure should rise, because of the encroachment upon the balloon by the bolus.

b. The preliminary fall in tone is followed by a gradual rise to a high level, and by the appearance of sharp, frequent contractions of varying intensity. This tremendous increase in tone and in activity continues for an interval of thirty to sixty minutes (longest seventy minutes), then suddenly ceases.

Washing out the stomach or inducing vomiting at this time shows that practically none of the meat has passed out of the stomach, nor has it been digested.

c. A fall in tone and in activity occurs suddenly, the period of quiescence showing rhythmic variation in tone, in the form of shallow waves described by Carlson as the normal state after a meal. Single contractions occur at

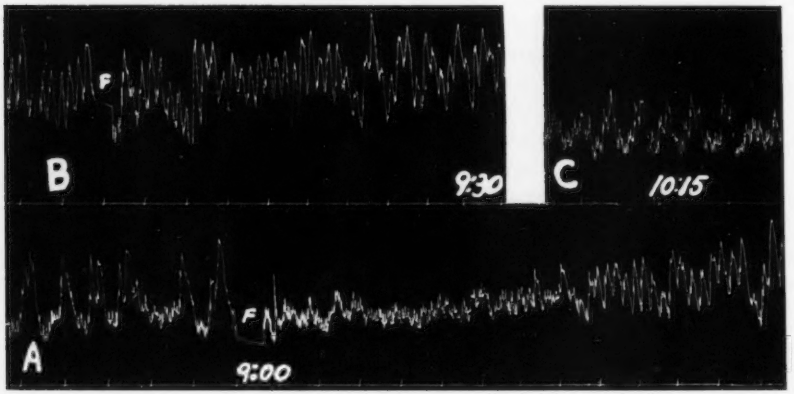

Fig. 1. Dog. Chloroform manometer record of gastric contractions. *A*, from left to right—hunger contractions. At *F* fed 50 grams meat. *B*, shows well-developed post-feeding activity; at *F* fed 50 grams meat. Note lack of effect. *C*, shows curve one hour and fifteen minutes after first feeding. Most of meat is still in the stomach. Time—one minute.

varying intervals which resemble empty stomach contractions. They are about ten to twenty seconds in duration and recur at intervals of one to six minutes. This phase lasts for at least three hours, the longest period followed.

A second meal given during the height of gastric tone and activity, *b*, had no effect whatever, and the graph continued its usual course (fig. 1 B) Food given during the quiescent period, *c*, failed to modify the graph.

It was found further that bread moistened with water, fed in amounts of about 50 grams, as of meat, had an effect similar to that produced by the meat. Further, meat suspended in water and squirted through the fistula caused the same increase in activity as that fed by mouth. Water

or 0.9 per cent salt solution given through the fistula, or water by mouth had no power to reproduce the meat curve. Twenty cubic centimeters of 50 per cent glucose solution given by tube through the fistula depressed the "hunger" activity of the stomach without subsequently augmenting its movements.

The period of increased activity, *b*, following the intake of food resembles closely the rise in tone and increase in rate of contraction which appear after the administration of insulin. About one hour after the subcutaneous injection of an adequate dose of insulin (one unit per kilo) there occurs a sort of gastric tetanus, graphically indistinguishable from that under consideration, and shown in figure 2 (author's unpublished experiments) (Bulatao and Carlson, 1924). Though the insulin curve resembles type III curve of Carlson's classification, it differs from it in that it is not influenced by the ingestion of food (fig. 2 A), the type III curve behaving

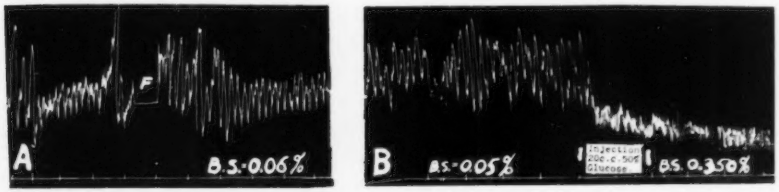

Fig. 2. Dog.. Chloroform manometer record of gastric hunger contractions. *A*, from left to right shows contractions one hour after 10 units of insulin subcutaneously. *F*, fed 50 grams meat. Note lack of effect. Compare tracing with figure 1 *B*. *B*, curve as for *A*. It shows effect of the intravenous administration of 20 cc. 5 per cent glucose solution. Time—one minute.

as described above for all "hunger" contractions (fig. 2 A and fig. 3 A). It differs from the "after feeding curve" described above in that it is destroyed by the intravenous administration of glucose solution (fig. 2 B) (1 cc. of 50 per cent solution per kilo), while neither the hunger curves nor the "after feeding curve" are so influenced.

Discussion. When food is put into the empty stomach of a healthy adult dog under the conditions of this experiment, the activity of that organ is markedly altered. The preliminary depression of the gastric hunger contractions and tone is interpreted as reflex inhibition, analogous to that obtained by stimulation of the mouth or esophagus.

The increase in tone and in activity following the preliminary depression has been hitherto interpreted as the digestive or activity phase of the stomach. What is its cause? Simple distention of the balloon, water or saline in bulk equal to the food eaten do not elicit this increase in activity. Both meat and water-moistened bread bring about the change. The sug-

gestion is that the stimulus is not chemical in nature, or if chemical, it is not specific. The experiments performed rule out a psychic influence, since meat eaten by the animal or administered by tube had identical effects.

Many writers on the subject have called this increase in activity a "digesting curve." The writer (1926) has gone so far as to expect that the period of activity here defined really represents the "emptying time" of the stomach. That this view is no longer tenable is brought out by these facts:

1. The increased activity begins immediately; reaches its height just as chemical digestion is beginning and comes to an end at the height of the chemical digestion.

2. At the end of the period of increased activity, it is found that practically all of the ingested material is still in an easily recognizable, partly undigested form.

The tracing of the increased activity following the ingestion of food so markedly resembled Carlson's type III hunger curve, that the writer thought the tracings were identical. To test this hypothesis it becomes necessary to reconsider what a hunger contraction is, and to find some way of distinguishing it from tracings of gastric activity elicited by other means. We have already seen that hunger activity and tone are depressed by the ingestion of food. Type III gastric tetanus is no exception to this rule. However, the tone and activity increase following the intake of food is not depressed by further food (50 grams), but is left unaffected.

It seems just to assume that the distinguishing feature between empty stomach contractions and other forms of gastric activity is the suppression of the former by food, followed by an increase in tone and activity, which is no longer influenced by food. The graph can determine only the degree of activity, never its origin. To the physical description of the activity of the empty stomach, so accurately defined by Carlson (1913), must be added the following qualifications:

a. Hunger contractions of the stomach of types I, II or III are suppressed by the ingestion of small amounts of food (about 50 grams of lean meat), and the gastric tone slightly lowered, if originally low (types II and III). The depressions last from one to two minutes.

b. The preliminary depression is followed in healthy animals by an increase in activity and tone.

No matter how closely the tracings of the activity of the stomach resemble so-called "hunger contractions," the writer feels that one is not justified in so designating the tracings, unless they meet the qualifications set forth in this communication.

Careful examination of the graphs shows that the activity here described to follow the ingestion of small amounts of food after a short preliminary depression, is not a continuation of the previous activity, but a new type

of activity. This is confirmed by the fact that the new curve is unlike the one it replaces in every type of tracing, save type III, and that it is just as easily elicited upon a quiescent organ. Moreover, the gastric activity following the ingestion of food is not "hunger" activity, because now no further change can be elicited by more food. Figure 1 C shows a type of gastric activity which might be interpreted as being weak hunger contractions of type I. The writer made that mistake in 1926 when he wrote: "The 'emptying time' considered here is that interval included between the disappearance of the hunger contractions due to feeding 50 grams of chopped meat and the re-appearance of those waves, the balloon remaining in situ throughout the observation" (p. 164). *The response of an organ to a physiological stimulus is a better index of the physiological condition of that organ than is its mere behaviour at any particular time.*

The accompanying tracings show that the first response of a "hungry" stomach to food is an inhibition of its activity and a fall in its tone. Were it to continue in such a state of inactivity and atonia, the individual would either feel satiated and discontinue eating too soon to meet his caloric needs, or the impulse to continue the intake of food would of necessity have to be continued by some non-gastric mechanism. The tracings, however, show that the stomach is not a passive bag into which food is thrown for no obvious reason. The preliminary fall in activity and in tone is soon followed by a period of increased activity and tone, coming on so soon after feeding, that the conclusion seems justified that it does not represent an attempt of the stomach to empty itself. It comes to an end at the height of gastric digestion (one hour after feeding) at a time when actual observation shows the food is still in the stomach. It apparently does not belong to the so-called "digestive" phase of gastric physiology. If so, what is its function and upon what conditions does it depend?

Might not the increase in concentration of either ion of hydrochloric acid in the blood cause the formation of more hydrochloric acid in the gastric glands and bring about this change in activity? The injection of acid is too disturbing to the animals. In order to change the Cl-ion concentration, the animals were injected intravenously with 1.0 cc. of 20 per cent sodium chloride solution per kilo of body weight. There followed no general disturbance. The animals would occasionally whine, but would soon quiet down again. Ten minutes after the injection the blood hemoglobin was found from 4 to 10 per cent below the pre-injection norm, but returned to normal at the end of twenty minutes.[2]

Following soon upon the injection of the hypertonic salt solution (fig. 3A), the gastric activity and tone began to increase and so closely

[2] The hemoglobin content of the blood was determined by comparing colorimetrically samples of blood diluted with 0.1 per cent HCl, considering the pre-injection sample as 100 per cent.

resembled the changes following feeding, that it was thought the mechanism for the latter had been discovered. However, similar doses of the solution given to a dog with a gastric Pavlov pouch failed to cause any appreciable secretion of acid juice after a test meal.

That the increase of chlorid-ion concentration of the blood and therefore of the gastric glands is not the cause of the gastric activity in question is clearly shown by the fact that the same gastric effect follows the intravenous injection of 1.0 cc. of 16 per cent Na_2SO_4 per kilogram of body weight. These "salt" tracings are unaffected by food; they persist after the hemoglobin and the chlorid-ion content of the blood have returned to normal; they can be obtained in animals (cats) which have been decerebrated and despinalized; and they are abolished by atropine.

Whatever the genesis of the increased tone and activity of the stomach after feeding, the conclusion seems justified that this increased activity has little or nothing in common with the ultimate process of digestion and

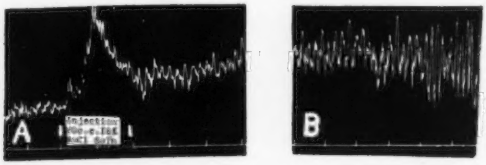

Fig. 3. Dog. Chloroform manometer record of gastric hunger contractions. *A*, from left to right, quiescent stomach. It shows immediate effect of the intravenous injections of 20 cc. of 16 per cent NaCl solution. Note rising tone. *B*, shows curve one-half hour after the salt solution injection. Compare curve with that in figure 1 *B* and figure 2. They are indistinguishable. Time—one minute.

expulsion of the chyme from the stomach. "Hunger," says Carlson, "may be considered as the signal that the stomach is contracted for action. The unpleasantness of hunger leads to eating; eating starts gastric secretion, distends the contracted organ, initiates the movements of gastric digestion and abolishes the sensation" (of hunger). It takes time to eat a full meal,—one-half to one hour. If the above quotation were strictly true, the ingestion of part of a meal would satisfy and the sense of hunger would be lost. This however is contrary to the common experience, that the impulse to ingest food increases with the first part of the meal. There is no obvious reason why the increased activity described above should not break through to consciousness and be felt as increased hunger.

Accordingly, the hunger mechanism may be divided into two phases:

a. *The empty stomach phase* with its three types of contractions, described by Carlson, and which are destroyed by food.

b. *The pre-digestive phase* which consists of increased gastric tone and activity, and is unaffected by the ingestion of a moderate amount of food.

It runs its course in from one-half to one hour, varying in duration in different individuals, and in the same individual at different times. It is abolished by atropine.

The pre-digestive phase suddenly ceases, the tone falls and there are only occasional contractions. Soon there appear tone waves, upon which are superimposed single contractions, which resemble those of the empty stomach. These may be hunger contractions. However, the stomach still has as much food in it as before. Food now given does not depress this activity, nor does it cause contractions resembling those of the pre-digestive phase. These then are not hunger contractions. This period is consequently designated as the digestive phase of gastric activity, marked by a, low tone with shallow tone rhythm, b, occasional contractions of type I, c, non-responsiveness to food, d, full chemical evidence of digestion.

SUMMARY

1. The work here reported was done on ten dogs with over a hundred observations.

2. An increase in activity and in tone of the stomach of dogs, following soon upon feeding, is described. This increased activity lasts from one-half to one hour. Its significance is discussed and the conclusion is reached that it belongs to the hunger, rather than to the digestive mechanism.

3. A definition of gastric hunger activity, adding a behavioristic to Carlson's physical description, is formulated.

4. The observation of Hughson and Scarff (1924), that hypertonic salt solution injected intravenously causes an increased activity of the gastro-intestinal musculature of cats under ether narcosis, is confirmed for the stomach of unanesthetized dogs.

5. Bulatao and Carlson's observations (1924), that insulin causes hyper-activity of the dog's empty stomach, is confirmed.

Part of the work here reported was completed under the Department of Pediatrics and part of the expense was met by the Institute of Infant Welfare, at the University of Minnesota. The writer gratefully acknowledges his debt to these departments in making this work possible. Thanks are also due to Professors C. C. Lieb and A. J. Carlson for their suggestions and criticism in the writing of this report.

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STUDIES ON DIABETES INSIPIDUS

II. The Diuretic Substance, Preliminary Observations[1]

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Investigators have long suspected that the diuresis of clinical diabetes insipidus is of humoral origin. Bailey and Bremer (1921) and Camus and Gournay (1924) have demonstrated that the diuresis of experimental diabetes insipidus, which many believe to be identical with clinical diabetes insipidus, is of humoral origin. There is however no satisfactory explanation of the nature of the humoral change in either clinical or experimental diabetes insipidus.

The possibility that it is due directly or indirectly to an upset in the functioning of the hypophysis is still disputed. One group of investigators concludes that there is no evidence that the hypophysis is involved since experimental diabetes insipidus may be produced without apparent injury to the hypophysis and fails to develop after hypophysectomy unless the floor of the third ventricle is injured. A second group of investigators refuses to accept this evidence arguing that in the first instance there may be injury to the infundibulum or to the circulation, which would prevent the absorption of the hypophyseal hormones, and in the second instance that there is not sufficient evidence that the gland was completely extirpated. Geiling (1926) reviews the literature relating to this phase of the problem.

Regardless of whether or not a disturbance in the functioning of the hypophysis is a causative factor, certain European investigators, who have demonstrated abnormalities in the water and mineral content of the blood of patients suffering from diabetes insipidus, have concluded that the diuresis proper is due to an upset in the exchange of water and crystalloids between the blood and tissues. Bauer and Aschner (1920), who review the literature and confirm the facts, state however that these changes are secondary to the water diuresis, which characterizes the condition, and that they develop in a normal individual who ingests similar quantities of water. Essentially the same conclusions may be drawn from the work of

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

Christie and Stewart (1917, 1922) who found no abnormality in the electrical conductivity of the blood or in the relative volumes of the corpuscles and serum before or after water deprivation in two patients with diabetes insipidus, and that such changes as occurred in the case of a third patient were within the limits produced in normal individuals by the ingestion of as great quantities of water.

The same criticism has been made of the suggestion of Camus and Gournay (1924) that the diuresis is due to an upset in purin metabolism. These investigators base their suggestion on the observation that there is an abnormality in the purin content of the urine in both clinical and experimental diabetes insipidus characterized by a decrease in the total quantity of uric acid and an increase in the total quantity of the other purins of the urine and of the phosphates, which is in proportion to the intensity of the diuresis. Kayser and Breton (1925) confirm these findings but state that the ingestion of similar quantities of water by a normal individual will produce the same changes.

Other investigators, among them Curtis (1924), conclude that diabetes insipidus is a primary thirst phenomenon. Certain facts established with regard to both clinical and experimental diabetes insipidus however would seem to contradict such a possibility. For instance the diuresis has been found to continue after water deprivation until definite symptoms of desiccation have developed in dogs suffering from experimental diabetes insipidus (Bourquin, 1927) and in a case of clinical diabetes insipidus (Bauer and Aschner, 1920); diuresis will develop in dogs operated for experimental diabetes insipidus independently of the preceding water intake (Roussy, 1925; Bourquin, 1927); and decerebrate pigeons injured in the region of the hypothalamus become rapidly desiccated by diuresis (Rogers, 1923).

The results reported in the first paper of this series led to the conclusions that experimental diabetes insipidus is caused by an irritation of the mammillary bodies and that the diuresis is due to a substance produced at the site of causative disturbance, which acts as a diuretic through the blood in either a direct or an indirect way. The present investigation was undertaken in the effort to detect the diuretic substance in extracts of the mammillary bodies, in the blood, and in the urine of dogs with experimental diabetes insipidus, and to determine its properties and mode of action.

METHODS. *Preparations of blood.* Blood was drawn from the carotid artery under novocain anesthesia. The greater portion of the proteins was precipitated by passing it into 2 volumes of 95 per cent ethyl alcohol containing 1 per cent of sulphuric acid. The precipitated proteins were collected on a filter and washed twice with distilled water. The combined filtrates were then freed of alcohol by distillation, made neutral to litmus with barium hydroxide, filtered, and concentrated to a volume of about

100 cc. This preparation was divided into several equal portions, each of which represented a known quantity of whole blood. In case the blood had been drawn from a non-diabetic dog, each portion was adjusted to a volume of 20 cc. and injected without further treatment. In the case of blood drawn from dogs suffering from experimental diabetes insipidus, one portion, called for convenience protein free blood concentrate, was likewise kept for injection without further treatment. The remaining portions were prepared in one of the following ways.

In 5 experiments phosphotungstic acid was added to one portion made 5 per cent acid with sulphuric acid. The precipitate and filtrate respectively were freed from sulphuric and phosphotungstic acids by barium hydroxide in the usual manner. The resulting solutions were neutralized with sulphuric acid, filtered, and adjusted to a volume of 20 cc. In 5 experiments a portion was diluted to approximately 200 cc., made just alkaline to litmus with barium hydroxide, boiled for determined periods of time, and then reneutralized and adjusted to volume. In 4 experiments a portion was carefully evaporated to dryness and well charred. The charred mass was ground in a mortar, redissolved in 100 cc. of boiling water, filtered, and adjusted to volume.

These preparations were kept on ice or evaporated to dryness and placed in a desiccator until needed for injection. The diuretic properties were tested by injecting them intravenously into normal dogs, the same dog being used to test the preparations made from the blood of any given diabetic dog for the purpose of comparing their activity (table 1). As a routine procedure the dogs were deprived of food for twelve hours preceding the injection and the urine was collected through a retention catheter for a control period of one hour before the injection. At least 6 hours, and usually 24 hours, were allowed between injections, this precaution being necessary for two reasons. In the first place when preparations of the blood of non-diabetic dogs were injected at two hour intervals, the second injection produced diuresis though the first had been without effect. In the second place it was found that a diuretic injected before the diuresis, which had been produced by the injection of blood from a diabetic dog, was fully over caused a diuresis far in excess of that which would normally be expected from it.

Brain extracts. The brains were removed as rapidly as possible under light ether anesthesia. Two blocks of tissue were cut from each brain, the first comprised as nearly as possible of the mammillary bodies only, and the second of a block three to five times as large taken at random from some other portion of the brain.

In one group of experiments a crude extract was made of each block by grinding it with sand and 5 cc. of 0.9 per cent solution of sodium chloride made 1 per cent acid with acetic acid, diluting with distilled water and ex-

TABLE 1

Protocols of representative experiments showing the comparative diuretic activity of the blood preparations

Each preparation was concentrated to a volume of 20 cc. for injection

DATE OF INJECTION	BLOOD PREPARATIONS					RECIPIENT OF INJECTION, DOG NO.	RESPONSE TO THE INJECTION IN TERMS OF CC. OF URINE PER HOUR AFTER INJECTION / CC. OF URINE PER HOUR BEFORE INJECTION		MANIFESTATIONS OF THIRST
	Donor, dog number	Condition of donor	Cc. per kilo of whole blood in the injection	Age of the preparation, days	Type injected		Hour		
							1	1 and 2	
March 22	102	Normal	128.4	4	Protein free blood concentrate	1	1.7		−
April 5	104	Diabetic	55.0	3	Protein free blood concentrate	1	3.1	3.6	+
April 6	104		55.0	4	Phosphotungstate ppt.	1	1.0	1.0	−
April 6	104		55.0	4	Phosphotungstate filtrate	1	15.0	14 0	++
April 30	107	Diabetic	55.0	7	Protein free blood concentrate	1	3.75	3.3	+
April 30	107		91.7	7	Phosphotungstate ppt.	1	1.0	1.0	−
May 1	107		55.0	8	Phosphotungstate filtrate	1	6.66	4.66	++
May 1	107		55.0	8	Phosphotungstate filtrate charred	1	2.5	2.4	−
May 19	111	Normal	73.3	3	Protein free blood concentrate	2	1.0	1.0	−
May 17	116	Normal	73.3	3	Protein free blood concentrate	2	1.3	1.3	?
May 21	121	Normal	91.7	4	Protein free blood concentrate	2	1.0	1.0	−
May 30	122	Diabetic	48.8	2	Protein free blood concentrate	2	4.0	3.8	?
May 31	122		48.8	3	The same boiled 20 minutes in alkaline solution	2	3.27	2.27	?
June 2	122		48.8	5	The same boiled 1½ hours in alkaline solution	2	1.75	1.4	?
June 11	140	Normal	68.7	3	Protein free blood concentrate	4	3.5	2.75	−
June 28	200	Diabetic	29.2	30	Protein free blood concentrate	4	4.5	3.9	+
June 29	200		29.2	30	The same boiled 1½ hours in alkaline solution	4	1.0	1.0	−

tracting for from 2 to 12 hours, boiling and filtering to remove the proteins, neutralizing to litmus with $\frac{N}{20}$ sodium hydroxide, and adjusting to a volume of 10 cc. A second type of preparation was made in another group of experiments by treating the crude extract with phosphotungstic acid in the same manner as the protein free concentrated blood, both the solution prepared from the precipitate and the filtrate being used for injection.

These preparations were injected into adult dogs weighing not over 10 pounds or into puppies weighing 7 to 8 pounds. In the latter experiments the urine was collected under barbital anesthesia through a rubber tube communicating by a glass cannula with the urinary bladder, which had been brought to an opening in the abdominal wall. An interval of three hours between injections was found sufficient in the use of these preparations.

Preparations from urine. Samples of fresh urine were collected from metabolism cages at two hour intervals. In one group of experiments they were prepared for injection by making them acid to litmus with sulphuric acid, evaporating to a small volume, extracting with ether, neutralizing to litmus with barium hydroxide, filtering, and adjusting to a volume of 20 cc. In another group of experiments phosphotungstate precipitates and filtrates were prepared for injection in the manner already described for blood preparations.

Results and discussion. *Brain extracts.* Extracts of portions of the brain other than the mammillary bodies were uniformly inactive in the case of both diabetic and non-diabetic animals. The crude extracts of the mammillary bodies of the brains of 4 out of 7 non-diabetic dogs were likewise inactive. Those of the 3 other non-diabetic dogs increased the urine output by an average of 1.66 times that of the control period for an average of 35 minutes. On the other hand similar extracts of the mammillary bodies of the brains of 12 out of 13 diabetic dogs produced an average increase in urine volume amounting to 3.15 times that of the control period and lasting for from 1 to $2\frac{1}{2}$ hours though only one-half of the entire extract was injected in 8 of the 12 positive experiments. The solutions of the phosphotungstate precipitates from the crude extracts of the mammillary bodies of the brains of 8 diabetic dogs and of the brains of 3 non-diabetic dogs were all inactive. The phosphotungstate filtrates from the crude extracts of the mammillary bodies of the brains of 5 of the diabetic dogs, on the other hand, produced a diuresis of the same order as that caused by the 12 crude extracts. The remaining 3 extracts and the extracts from the non-diabetic brains were not tested.

The diuresis, which is produced by these extracts, is similar to that which follows injections of Ringer's solution rather than to that following the injections of hypertonic salt solutions for it is typically a prolonged moderate diuresis of nearly the same intensity throughout the period

(fig. 1). The injection of a diuretic before the end of one of these periods was observed to result in a response much greater than such an injection ordinarily produces. The reason for this is not explained by these experiments but the fact is significant in that it is known that the injection, or the ingestion, of sodium chloride in clinical or experimental diabetes insipidus results in an augmentation of the diuresis beyond that which would normally occur.

These results would seem to justify the conclusions that there is a substance in the region of the mammillary bodies of the brains of dogs, or in the tissue immediately surrounding them, which possesses diuretic properties; that it is present in larger amounts in the mammillary bodies of dogs suffering from experimental diabetes insipidus than in those of non-diabetic dogs; that it is probably not present elsewhere in the brain in comparable amounts; and that it is not precipitated by phosphotungstic acid.

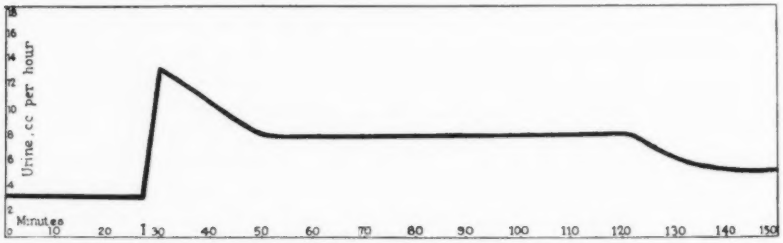

Fig. 1. The diuresis produced in an 8-pound puppy by the injection of the extract of the mammillary bodies from the brain of a 30 pound dog passing 100 cc. of urine per hour. *I*. Time of injection.

Preparations of blood. The protein free blood concentrates of 9 non-diabetic dogs injected in an average quantity of 68.2 cc. per kilo produced an average augmentation of the urine output of 1.2 times that secreted during the control period for the first hour following the injection, and of 1.1 times for the first two hours. On the other hand the injection of the protein free concentrates from 11 diabetic dogs in an average amount of 52.14 cc. per kilo produced an average increase in the urine output of 4.99 times that secreted during the control period for the first hour following the injection, and of 4.39 times for the first two hours (tables 1 and 2).

Solutions containing the phosphotungstate precipitates from the protein free blood concentrates were uniformly inactive (5 experiments), while solutions containing the phosphotungstate filtrates from the same were much more active than the corresponding protein free blood concentrates (5 experiments) (tables 1 and 2). Preparations which had been boiled while alkaline for periods of from 1 to 2 hours, had lost their activity in whole

or greater part (4 experiments), but boiling for 20 minutes while alkaline had diminished the activity of a fifth preparation only slightly as compared to the activity of the corresponding protein free blood concentrate. Charring uniformly decreased the activity of the preparation in 4 experiments (tables 1 and 2).

The curve of diuresis was of the same type as that produced by the injection of active extracts of the mammillary bodies and the diuresis was of the same order of intensity as that characteristic of dogs with experimental diabetes insipidus, which have been restricted to the water intake

TABLE 2

Summary of the results obtained in the experiments comparing the diuretic activity of blood preparations

NUMBER OF EXPERIMENTS	CONDITION OF THE DONOR OF THE BLOOD	TYPE OF INJECTION	AVERAGE NUMBER OF CC. PER KILO OF WHOLE BLOOD IN AN INJECTION	AVERAGE RESPONSE TO INJECTION IN TERMS OF AVERAGE CC. OF URINE PER HOUR AFTER INJECTION / AVERAGE CC. OF URINE PER HOUR BEFORE INJECTION	
				Hour	
				1	1 and 2
9	Normal	Protein free blood concentrate	68.2	1.2	1.1
11	Diabetic	Protein free blood concentrate	52.14	4.99	4.39
5	Diabetic	Phosphotungstate filtrate	35.2	6.37	7.08
5	Diabetic	Phosphotungstate precipitate	101.0	1.0	1.0
2	Diabetic	Boiled in alkaline solution 2 hours	35.2	1.0	1.0
2	Diabetic	Boiled in alkaline solution 1½ hours	48.8	1.75	1.4
1	Diabetic	Boiled in alkaline solution 20 minutes	48.8	3.27	2.27
4	Diabetic	Charred	55.88	2.36	2.0

normal for non-diabetic dogs. Pronounced thirst was manifest after the injection of active preparations, the dogs drinking large quantities of water as soon as allowed off the table. This was also true of dogs receiving injections of active extracts of the mammillary bodies in the survival experiments. This fact is of interest in that the actual loss of fluid to the animals had been relatively much less than that which is produced by the injections of hypertonic solutions of sodium chloride and sodium sulphate which it would be safe to use and which are not followed by manifestations of thirst (Brunn, 1925, and observations in this laboratory).

The results demonstrate that there is a diuretic substance present in the blood of diabetic dogs, but not in the blood of non-diabetic dogs in amounts which can be detected by these methods, which is similar to the diuretic substance in the extracts of mammillary bodies in so far as the two have been compared. The substance in the blood has the further characteristic, which may or may not be possessed by the substance in the mammillary bodies, of being destroyed by prolonged boiling in weakly alkaline solution, of being rendered less active when the preparation is charred, and of being stable over at least 8 days in neutral solution and over at least 30 days in the dry form (table 1). The fact that the phosphotungstic filtrates are relatively more active than the corresponding so-called protein free blood concentrates is perhaps in line with the findings of cross-transfusion experiments between normal dogs and between a normal and a diabetic dog. In these experiments periods of cross transfusion as short as 1½ minutes produced a relative oliguria or even anuria (Bourquin, 1927) presumably due to the effect of some protein on the renal epithelium or renal circulation, for the so-called protein free blood concentrates, from which most though not all of the proteins have been removed, have no such action.

Preparations of urine. A large series of urine preparations of both types proved uniformly negative, results which these experiments fail to explain. It may be that the diuretic substance of the blood acts indirectly to produce diuresis, or that it is labile in the urine, or that it is secreted in such small amounts in a 2 to 4 hour period that it cannot be detected by the methods thus far used.

SUMMARY

A substance having diuretic properties has been extracted from sections of the brains of dogs, which were cut in such a way as to include only the mammillary bodies as nearly as that is possible. This substance is present in much larger quantities in the mammillary region of the brains of dogs suffering from experimental diabetes insipidus than in the same region of the brains of normal dogs, and has not been found in extracts of other regions of the brain. It is not precipitated by phosphotungstic acid.

A substance having diuretic properties has also been demonstarted in the blood of dogs suffering from experimental diabetes insipidus but not in the blood of normal dogs, which is less active after the preparation has been charred, which is destroyed by prolonged boiling in a weakly alkaline solution, which is relatively stable in neutral solutions and in the dry form, and which is not precipitated by phosphotungstic acid.

The substances in the mammillary region and in the blood both cause the same type of diuresis, both cause an excessive response to diuretics injected

during the period in which they are acting, and both produce manifestations of thirst.

Efforts to detect a similar diuretic substance in the urine have been unsuccessful.

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FURTHER STUDIES ON THE ALLEGED INTERRELATIONSHIP OF THE PANCREAS AND THE THYROID

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The maintenance of a nearly uniform concentration of sugar in the circulating blood is a fundamental adjustment of the human body. Normal accumulation of sugar doubtless depends on a variety of factors, among which the integrity of the pancreas, though very important, does not stand alone, and the disturbances of the carbohydrate metabolism may be the result of conditions affecting other organs.

That the thyroid may have relationship to the carbohydrate metabolism, and that the pancreas has intimate relationship with it is well known, but the experimental evidence concerning such a possible relationship is very conflicting. It was because of conflicting data that this investigation of the alleged interrelationship between these two endocrine organs was undertaken.

It seemed more feasible, however, to test out the effect of feeding moderately large doses of desiccated thyroid extract to dogs in which a pancreatic deficiency had been artificially produced by the removal of most of that gland. Under such experimental conditions an experimental hyperthyroidism (Kunde) might suggest an interference with normal carbohydrate metabolism either by an elevation of the blood sugar with or without an attendant glycosuria. In order to control the effect of an elevation in temperature itself (a condition which commonly accompanies the feeding of moderate doses of desiccated thyroid extract) an artificial hyperpyrexia was induced by injecting a number of dogs with sodium nucleinate. To control the effect of heightened basal metabolism due to thyroid feeding, quinine was fed to a number of dogs. It was by these methods that we hoped to show whether a direct or indirect relationship existed between the thyroid and the pancreas or whether other conditions had an influence in altering the carbohydrate metabolism of the animal.

LITERATURE. On reviewing the literature concerning the possible existence of a relationship between these two endocrine organs one is struck by the many conflicting reports in both the clinical and the experimental investigations.

It was considered common knowledge that in exophthalmic goiter

(hyperthyroidism) there is a lowered tolerance to carbohydrates, while in myxedema and cretinism there is an increased sugar tolerance. Dumontpallier (1867), Jones (1893), Fr. Muller (1906) and McLean (1922) reported glycosuria in patients taking thyroid in large amounts. Kraus and Cramer (1912) found that after thyroid ingestion, the liver does not retain glycogen as well as before. Friedman and Gottesman (1922) contended that they had obtained experimentally a diminution in the glycosuria of pancreatic diabetes in dogs following thyroidectomy. Fitz (1921) attributes the lowering or diminution in glycogen to a change in the rate of metabolism and not to the fact that a portion of the gland is functionless. Wilder (1926) corroborates Fitz to the effect that the evidence clinically is not indicative of the curative effect of thyroidectomy in diabetes. Wolfson (1926) proceeded on the same general line of investigation that Friedman and Gottesman (1922) had followed, but by using Luckhardt's method of combating parathyroid tetany, prolonged the life of his totally depancreatectomized and thyroidectomized dogs. The negative conclusions reported concerning such a possible relationship had a better experimental basis than the work reported by Friedman and Gottesman.

Methods. The procedure in these experiments was to place the dogs on a standard diet of 250 grams of beef heart and 225 cc. of milk. The control diet was not changed during the course of the experiment. The dogs were placed in metabolism cages. Total output of urine per twenty-four hours was recorded; and samples were then taken from the daily output for analysis. Toluol was used as a preservative to minimize bacterial decomposition. At the time the urine samples were taken, 5 to 6 cc. of blood were drawn from the dog's saphenous vein and put in tubes containing powdered potassium oxalate as an anticoagulant.

To test qualitatively for urinary sugar, the Fehling or Benedict tests were used. If the tests were negative the tests were discarded; if positive, the amount was determined by the method of Folin-Wu, two determinations being made on each sample.

For blood sugars, the Folin-Wu quantitative method was also used; three to four samples of each dog were run daily, the average being recorded as expressing the blood sugar level for that day.

Control experiments were run on the animal for three days or upward before preparing it for the initial experiments.

In performing a partial pancreatectomy, approximately seven-eighths of the pancreas was removed, the remnant being connected to the duodenum through the duct of Santorini. In the total pancreatectomy the duct of Santorini and Wirsung was ligated, and the pancreas was stripped off, care being taken not to leave any remnant.

In trying to bring about the semblance to a hyperthyroid state, the dos-

age of 0.5 to 0.8 gram per kilo, as recommended by Kunde, was tried and found to have the desired effect. The desiccated thyroid extract was mixed with the animal's food.

Ten to fifteen cubic centimeters of a 10 per cent solution of sodium nucleinate were injected subcutaneously to induce a high fever (hyperpyrexia).

The amount of quinine fed was from 0.25 to 0.35 gram per kilo, as recommended by Tatum. This was given by stomach tube, after which the animal was allowed its food.

RESULTS. I. *The effects of thyroid medication on the blood and urinary sugar of normal and partially pancreatectomized dogs.* *a.* No effects on the blood sugar were noticed on feeding *normal* dogs with small or large quantities of desiccated thyroid (0.5 to 0.8 gram per kilo).

DOG	BLOOD SUGAR			TEMPERATURE	
	Average	Low	High	Normal	After feeding thyroid
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*		
I	91	84	95	101.8°	102.6°
II	98	94	101		
III	94	92	96		

b. On feeding 0.5 gram thyroid extract per kilo body weight to partially depancreatectomized dogs, a mild hyperglycemia was noticed. The increase in blood sugar began to appear two days after the first thyroid was fed and reached its height on the seventh day.

DOG	BLOOD SUGAR			TEMPERATURE (NORMAL)
	Average	Low	High	
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
A	88	80	96	101.4
B	98	95	100	101.0
C	91	88	93	101.8
After feeding				
A	111.9	95	125	102.1
B	108	102	119	101.7
C	115	104	125	102.6

c. It was apparent that the high protein control diet plus the thyroid feeding could not entirely break down the reserve of the intact pancreatic remnant. To overthrow this balance, it was decided to overtax the remaining pancreas by adding known amounts of sugar or bread to the control diet; then on subsequent thyroid feeding (0.8 gram per kilo), a greater hyperglycemia and possibly a glycosuria should result.

DOG	BLOOD SUGAR			TEMPERATURE
	Average	Low	High	
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
A	88	80	96	101.4
B	98	95	100	101.6
C	91	88	93	101.8
		After feeding		
A	118	117	122	
B	115	112	119	
C	161	123	184	

d. After continued feeding of thyroid extract a hyperglycemia and a mild glycosuria developed.

DOG	BLOOD SUGAR			TEMPERATURE
	Average	Low	High	
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
A	139	126	174	
B	137	132	173	
C	148	123	184	
		After feeding		
A	329	292.5	665	102.5
B	664	444	1080	103.1
C	469	289	778.3	102.6

As a control to the hyperpyrexia that is brought about by thyroid feeding, subcutaneous injections of a 10 per cent sodium nucleinate solution were given every two days.

II. *Effects of sodium nucleinate injections on the blood sugars of normal and partially pancreatectomized dogs.* *a.* Subcutaneous injection (10–15 cc.) of a 10 per cent sodium nucleinate solution into normal animals yielded a mild hyperglycemia.

DOG	CONTROL BLOOD SUGAR			TEMPERATURE	
	Average	High	Peak	Normal	After injection
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*		
A	92	96	108	102°	103.8

b. Subcutaneous injection (10–15 cc.) of a 10 per cent sodium nucleinate

solution into partially pancreatectomized dogs caused a more marked hyperglycemia than in *a*.

DOG	AVERAGE BLOOD SUGAR	PEAK—AFTER INJECTION	PEAK	TEMPERATURE	
				Normal	Injection
	mgm. per 100 cc.				
A	95	96	137	102	104
B	86	91	132	101.3	104.5
C	83	87	112	102.9	103.4

III. *Effect of oral administration of quinine sulphate on the blood sugar of normal and completely pancreatectomized dogs.* To produce a desired lowered metabolic rate quinine sulphate was fed orally.

a. On feeding 0.25 to 0.35 gram per kilo of quinine sulphate by stomach tube to a normal animal a mild hyperglycemia resulted.

DOG	AVERAGE BLOOD SUGAR	PEAK	AFTER FEEDING—PEAK	TEMPERATURE	
				Normal	Feeding
	mgm. per 100 cc.				
I	88	89	127	101.7	101.9

b. On feeding 0.25 to 0.35 gram per kilo of quinine sulphate by stomach tube to totally depancreatectomized dogs there did not occur any arrest in the hyperglycemic and glycosuric trend.

Discussion. Glycosuria following the therapeutic use of thyroid extract has been observed by Opie, and Kraus and Kramer (1912), but in none of the cases associated with exophthalmic goitre has the condition of the pancreas been studied.

In producing a partial pancreatectomy in which seven-eighths of the pancreas was removed the animal was brought to a border-line state. On subsequent feeding of thyroid extract (protocol Ib) a hyperglycemia developed, slowly at first, but on repeated feeding a glycosuria developed. On adding 50 grams of bread to the diet of dogs A and B, and 2 grams of dextrose to the diet of dog C, we prevented an extreme emaciation from taking place in our dogs, for they were beginning to lose weight rapidly by use of their body proteins and fat. During the feeding of the desiccated thyroid extract the dogs were very polyphagic, and in one part of the experiment dog B delivered four pups. A marked but temporary glycosuria developed (1.08 gram per 24 hours). Carlson and Drennan (1911) observed that glycosuria did not follow in the pregnant dog after removal of the pancreas, until the pups were born. This was confirmed by others and it explains the fact that the fetal pancreatic hormone supplied the deficiency in the mother.

Thyroid feeding to an animal with an intact pancreas had no effect despite the fact that a hyperpyrexia was induced. For that purpose Doctor Kunde turned over to us two dogs. One (I) had been thyroidectomized and fed 5 grams of thyroid extract per day since February 2, 1926, and about ten determinations were run on his blood sugar, and at no time did he exhibit a hyperglycemia or glycosuria. The other dog (II) had been fed thyroid extract (20 grams per day) since August 1, 1926, and he, too, did not show signs of a hyperglycemia and glycosuria.

Sodium nucleinate is a protein which when injected produces a febrile state. On injecting it subcutaneously it was noticed that a mild hyperglycemia was produced in the normal animal with an intact pancreas, while the thyroid extract did not have such action. In partially depancreatectomized dogs, the hyperglycemia mounted a little more rapidly than in the normal animal. The hyperglycemia was not carried to the glycosuric stage. Nord (1927), too, had found that parenteral injections of Witte's peptone, glycocoll, glutanic acid, produced a hyperglycemia in the animal.

Quinine sulphate, a drug which according to some supposedly lowers the basal metabolism in an animal, had no effect in arresting the hyperglycemic and glycosuric trend. Tatum had found previously that the administration of quinine sulphate to normal rabbits produced a hyperglycemia. Our results confirm his finding and that of Silberstein (1915) in the normal dog. The animals that subsequently died from administration of quinine were found at autopsy to have a focal necrosis of the liver probably due to the administration of that drug.

SUMMARY

1. The feeding of thyroid to an animal with an intact pancreas produces no effect on the blood sugar.

2. The feeding of thyroid to an animal on the border-line (partially pancreatectomized dog), leads to a hyperglycemia, and a glycosuria associated with a fever.

3. Sodium nucleinate when injected subcutaneously produces fever, causes a hyperglycemia in the normal animal whose pancreas is intact.

4. Quinine, a drug which presumably diminishes the basal metabolic rate of an animal, has no effect on the hyperglycemic trend of a diabetic animal. Hyperglycemia is even produced in the normal dog by its administration.

CONCLUSIONS

1. The hyperglycemia and glycosuria which follow thyroid medication are for the most part due probably to the increased basal metabolic rate which accompanies thyroid feeding; for the injection of sodium nucleinate

which produces an increased basal metabolic rate with fever produces a similar state of hyperglycemia. As far as these results go they indicate that the thyroid influences carbohydrate metabolism only indirectly through its effect on the basal metabolic rate.

2. On the basis of these experiments it is concluded that there is no direct but an indirect relationship between the thyroid and the pancreas.

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INSULIN ANHYDREMIA[1]

Importance of the Water-Reserve in a Physiological Crisis

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Pronounced hypoglycemia due to the injection of large amounts of insulin is associated with the development of anhydremia. This was originally observed by Drabkin, Page and Edwards (1) and has been confirmed by several investigators (Olmsted and Taylor (2); Villa (3); Klein (4); Levine and Kolars (5); Best and Ridout (6); Foshay (7)). In a final paper Drabkin (8) has criticized the opposing conclusions of Hamilton, Barbour and Warner (9) and Haldane, Kay and Smith (10). The latter investigators have withdrawn their original contention in a second paper (11). Drabkin (8) has again emphasized that the amount of insulin injected is an important factor, in that the use of "mass" doses of insulin is essential in this type of experiment; and he believes that the sudden, profound hypoglycemia—a virtual purging of the blood of its sugar—is the primary cause of the anhydremia.

The original investigators (1) observed that, although in insulin hypoglycemia blood concentration invariably occurred, the animals studied fell into two groups: Some developed anhydremia rapidly with an acute fall in blood pressure; a larger group exhibited a gradual rise in blood concentration with a well-maintained blood pressure, showing a tendency to return spontaneously toward normal. The clinical observations of Joslin, Gray and Root (12) indicate that desiccation through diarrhea is an important factor in rendering diabetic children especially prone to dangerous insulin hypoglycemia. Voegtlin, Dunn and Thompson (13) have found that rats are more susceptible to insulin shock in hot weather than in cold.

The natural outgrowth of the problem was to study the influence of the water intake upon the type of reaction to insulin, especially with reference to the severity of the resulting anhydremia. A more exact statement of

[1] A preliminary report of this work has appeared in 1925, Proc. Soc. Exper. Biol. Med., xxii, 369. The subject matter of this paper has been used by H. Shilkret as a thesis in candidacy for the degree of Doctor of Medicine, Yale University, 1926.

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the problem was framed in the question: "Will an animal which has been desiccated through water starvation react differently to a large dose of insulin than one which has had a sufficient intake of fluid?"

Experimental procedure. Unanesthetized dogs (5 to 10 kgm. in weight) were used. They had been fed upon the Cowgill "synthetic" diet (14). Blood samples were removed mainly from the heart. After drawing preliminary "control" samples of blood, the dogs were either deprived of water or given by stomach-tube an average of 800 cc. of water daily for periods of 4 to 9 days. The preliminary treatment in one experiment was a *laissez faire* procedure—water *ad libitum* for 10 days. As an added check upon the results, one of the dogs previously water-starved was subsequently studied under a forced fluid régime, and then again subjected to water deprivation. Just before the intravenous administration of a large dose of insulin (20 units per kilogram body weight), the second "control" samples were drawn. Blood samples were then taken at suitable intervals following the insulin administration. Each blood sample was analyzed for hemoglobin and, in some of the experiments, for dry solids (as indices of the amount of blood dehydration) and for sugar. A modified Cohen and Smith (15) technique was employed for the estimation of the hemoglobin and the method of Shaffer and Hartmann (16) was used for the determination of the sugar.

A careful record was kept of the symptomatic responses and their nature. Under this head were noted such factors as the presence or absence, type and time of onset of convulsions, the time of onset of coma, the rapidity and type of recovery, if any, after the administration, in each case, of resuscitative quantities of glucose (150 to 200 cc. of a 10 per cent sterile solution intraperitoneally). In the case of the death of the animal a complete autopsy was promptly performed and the post-mortem findings carefully noted.

The experimental results. Depriving a dog of water for a period of 4 days caused *per se* only a negligible increase (2.9 per cent, experiment 1) in the concentration of the blood. Water-starvation for longer periods, however, resulted in a moderate concentration (12.6 to 17.4 per cent above the original values, experiments 4 and 5).

The response of the blood sugar to insulin was not influenced by the water intake. The desiccated animals reacted in this respect no differently than those which had received water. In each group the fall was rapid and to a low level (from the original values to 40–0 mgm. per 100 cc. of blood).

After the administration of insulin the previously desiccated animals developed a more profound concentration of the blood than the others. The values for blood concentration as determined by the dry blood solids content were approximately one-half those derived from the hemoglobin values. The essential data are summarized in the table.

All the dogs which were not deprived of water had, during the course of the hypoglycemic reaction, severe spastic convulsions. This group of animals recovered within approximately five minutes after the administra-

TABLE 1

EXPERIMENT NUMBER	PRELIMINARY TREATMENT	BLOOD SUGAR		HEMOGLOBIN			DRY BLOOD SOLIDS		LENGTH OF TIME AFTER INSULIN		INCREMENT IN HEMOGLOBIN	INCREMENT IN BLOOD SOLIDS	BLOOD CONCENTRATION‡	
		Before insulin	After insulin	Before water starvation	Before insulin	After insulin	Before insulin	After insulin					Calculated from hemoglobin increment	Calculated from blood solids increment
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	hr.	min.	per cent	per cent	per cent	per cent
* 1	Water-starved (4 days)	0.097	0.007	103	106	157	20.7	26.5	3	10	51	5.8	48.1	28.0
* 3	Forced fluids (5 days)	0.088	0.000		100	117	18.5	20.3	2	30	17	1.8	17.0	9.7
* 4	Water-starved (5 days)	0.106	0.040	103	116	190	21.2	28.3	3	1	74	7.1	63.8	33.5
5	Water-starved (7 days)	0.098	0.031	132	155	212	23.0	29.2	3	10	57	6.2	36.8	26.9
2	Forced fluids (7 days)	0.099	0.013		108	139	1.43†	1.73†	2	10	31		28.7	
6	Forced fluids (9 days)	0.091	0.028		104	132			2	22	28		26.9	
7	Water *ad lib.* (10 days)	0.093	0.026		116	145			3	9	29		25.0	

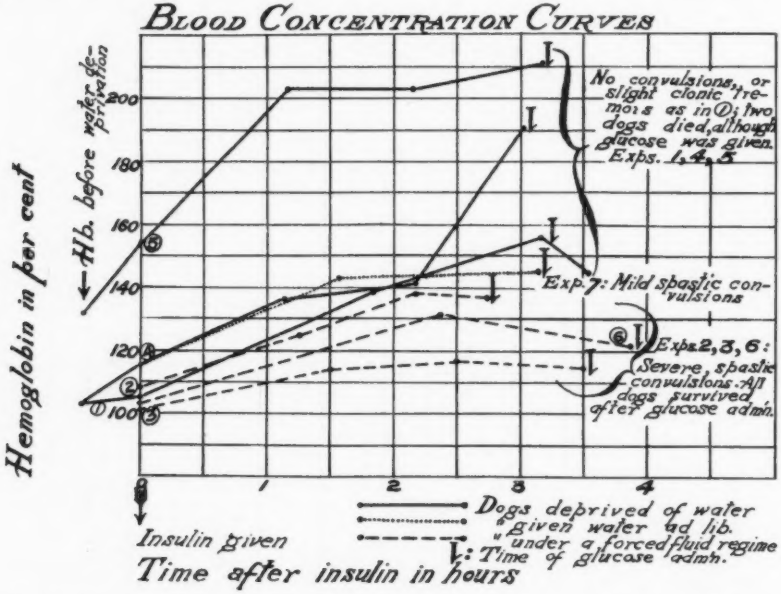
* Experiments performed upon the same animal.

† Relative densities of blood, determined gravimetrically. Calculated from these values blood concentration is 20.9 per cent.

‡ Some of these experiments have been cited by the senior author in another connection (8).

tion of glucose and stayed well. The most striking observation upon the desiccated animals was the total absence of spastic convulsions. This group of dogs had slight, generalized clonic tremors, resembling shivering movements. They passed rapidly (20 minutes to 2 hours) into a comatose

state and thence into complete unconsciousness. Arhythmia of the heart and complete dilatation of the pupils (probably evidences of medullary and cerebral irritation) were present here. The administration of glucose brought the animals out of coma, but in experiments 4 and 5 the dogs were too weak to stand and death came within 10 minutes and 5 hours respectively after the glucose injection. The dog which was used in experiment 4 was previously water-starved for a shorter period of 4 days. It survived this "milder" treatment only after a very stormy convalescence of two days, during which it voluntarily drank large quantities of water. The same animal was also studied under a forced fluid régime, experiment 3,



in which its recovery from insulin was rapid and uneventful. The findings at necropsy were without significance except, perhaps, for the apparent intravascular clotting of the blood.

The appended chart brings out interesting differences in the hemoglobin concentration curves of the two groups. The curves for the desiccated animals are steep and continuous; those of the others are more gradual in their ascent, with a tendency for a spontaneous return to the normal value during the third hour.

Discussion. The data furnish evidence of the influence of the existing state of hydration or water balance of the organism upon the severity of the response to an agent like insulin. The dogs which were previously

desiccated may well be considered to have been more susceptible to insulin. The differences in reaction of the two groups of dogs described by Drabkin and Edwards (1) may have been due to the chance desiccation of some of the dogs (all of which had water *ad libitum*). Such an assumption seems feasible in view of the similarity of the blood concentration curves recorded in the present paper with those in the earlier publication. This is interesting in emphasizing the idea that the state of hydration may be one of the factors in the phenomenon which, for lack of knowledge, we call susceptibility to a drug.

The dog, probably due to the lack of loss of appreciable amounts of moisture from the surface of the body, can withstand water deprivation for much longer periods than man (17). The moisture content of the Cowgill diet is low and, as might be expected, after 2 to 3 days of water deprivation the animals refused one-half or more of their allotted ration. Thus, in the latter days of the longer water-starvation experiments the dogs were living partially on the resources of their own tissues. By so doing the animals were making use of the so-called water of metabolism for excretory purposes, etc. In the exact sense of the term (17) we were not dealing with water hunger *per se*. The fact that such profound changes in the reaction to an agent like insulin followed water deprivation in a species which is thought to be particularly resistant to water-starvation is, therefore, especially noteworthy. Underhill and Kapsinow (18) have found that the administration of Witte's peptone or histamine to dogs which have been previously deprived of water does not result in the characteristic blood concentration response. Magnesium sulphate also failed to act in expected fashion when administered to water-starved animals. These findings were "interpreted as further proof of the large factor of safety existent in the body in the regulation of water equilibrium." The experiments reported in the present paper suggest that the "large" margin of physiological safety in reference to water balance in the organism may be more apparent than real. The production of anhydremia on top of desiccation is apparently incompatible with life.

The occurrence of convulsions may be related to the water content of the organism. Several experiments, performed by the senior writer (D.L.D.) in an attempt to establish a relation between intracranial pressure, type and time of occurrence of convulsions and the condition of the blood, have been inconclusive. In view of this it does not appear profitable to generalize at the present time concerning the cause of the absence of spastic convulsions in the desiccated animals.

SUMMARY AND CONCLUSIONS

The type of reaction to insulin is influenced greatly by the water-reserve of the animal. Dogs desiccated through water-starvation when given

large doses of insulin developed a more profound anhydremia than those which had water. Spastic convulsions were not observed in the desiccated animals although they appeared in those given fluids. The latter animals invariably recovered upon glucose administration, while those that were deprived of water for periods longer than 4 days did not survive.

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ABSORPTION OF INSULIN FROM THE ALIMENTARY TRACT OF DEPANCREATIZED DOGS, WHEN PROTECTED BY BLOOD SERUM

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This laboratory has reported from time to time (1) (2) (3) indisputable evidence of absorption of insulin from the alimentary tract. The concentration of insulin reaching the general circulation, however, has always been small compared with that which follows subcutaneous administration. Notwithstanding that huge doses have been given there has never been any evidence of hypoglycemia. To some extent this has been compensated for by a more prolonged effect, lasting indeed in some instances (2) into the second day after administration.

Recently advantage has been taken of the anti-tryptic effect of blood serum[1] to protect the insulin given by stomach tube[2] to depancreatized dogs from "pancreatin" given with the food. The first attempts to demonstrate the absorption of insulin when protected in this way having proved successful, the writers have followed up the subject now for nearly two years and are able to report convincing evidence of absorption under what might be supposed to constitute very adverse conditions. To maintain a depancreatized dog in good condition it is necessary to supply artificial pancreatic digestion. In this laboratory reliance has been placed in Merck's "Pancreatin." Even when as much as 30 grams of the dry powder were mixed with the day's food (eaten in two meals) dogs subsequently proved by thorough microscopic examination as well as by metabolic evidence to have been completely depancreatized have become sugar-free, have been kept alive for three and four months and at times have been made to gain in weight. The protocols of these dogs will be given after certain crucial experiments have been presented.

[1] The authors are indebted to Dr. Clyde C. Sutter of Rochester for calling their attention to the work of Jobling and Peterson (5) on the ferment-inhibiting substances of the blood from which, as well as from Effront's work (6), a number of valuable suggestions have been received.

[2] In administering the insulin by stomach tube two special precautions have always been observed; namely, to use a small stomach tube (large catheter) thereby avoiding emesis, and to leave no serum in the mouth, thereby avoiding absorption from the tongue or buccal mucous membrane.

EXPERIMENTAL. The best evidence of insulin action is from the respiratory quotient. A falling blood sugar may be very misleading, for blood sugar is usually falling slightly except within the first two hours or so after meal time and when only slight effects from alimentary insulin are expected, results are likely to be confusing. The respiratory quotient also normally is falling from about the same point after each meal. But the effect of insulin being to *raise* the quotient, even slight insulin effects may be detected. The D to N ratio in the writers' experience is less dependable with depancreatized dogs than with phlorhizinized dogs. Nevertheless even this criterion may be useful if employed with full realization of its limitations.

Evidence of increased combustion of sugar under the influence of insulin protected by blood serum is clearly to be seen in the following experiments.

Dog I. Female collie, weight 12.0 kgm.; pancreatectomy March 8, 1926. Dog kept under subcutaneous insulin treatment for 5 days until abdominal wound was well healed. No insulin on March 14 or 15. From March 16 to 19 inclusive the urine was analyzed for dextrose and nitrogen and on the 17th, 18th and 19th the dog was placed in the respiration chamber for determination of the respiratory metabolism. In this instance the dog had no food, other than the dextrose given, from the evening of March 15 (meat), until the evening of March 19. Two hundred clinical units of commercial insulin were given in blood serum on the 17th and 19th; none on the 18th.

On the 16th at 3:20 p.m. the D:N ratio was 2.56 and on the 17th just before ingestion of sugar it was 2.80. The dog therefore was in the typical state of diabetes following pancreatectomy and upon a meat diet. Without insulin the dog had excreted sugar on the 16th from 9:15 a.m. to 3:20 p.m., at the rate of 1.04 gram per hour, at the time when the D:N ratio was 2.56; and for the corresponding period on the 17th when the ratio was 2.80 the hourly excretion was 1.09 gram (see table 1). At this rate, if no dextrose and no insulin had been given, the dog should have excreted about 3.3 grams in the three-hour period immediately following. Actually the urine contained 9.6 grams or at least 6.3 grams of the ingested sugar. The dextrose given assayed 92.9 per cent pure d-glucose, making 18.58 grams. Hence a little more than 12 grams were retained; for the excretion the next period was even lower than before insulin; namely, 0.99 gram per hour. It does not seem possible that insulin given subcutaneously four days before could still be sufficient to cause this retention. In any case proof that a considerable spurt was given to the combustion of sugar by the alimentary insulin is seen in the R.Q. of 1.06 obtained in the second 45 minute period of the respiratory metabolism, followed by a sudden return the next period to basal. This is the usual behavior of the metabolism after a subcutaneous injection (7).

On the 18th the same dose of blood serum and dextrose but without insulin were given for control. There was apparently a stimulating effect

on the respiratory metabolism, but it was by no means so great as on the 17th. A true basal metabolism was expected. Whether the effect was due to insulin stored in the liver or elsewhere from the previous dose or was due to some insulin contained in the ox-blood serum itself cannot be decided in this case because the insulin and serum were given before the

TABLE 1

Dog I

DATE	TIME	TOTAL D	TOTAL N	D/N	D PER HOUR	N PER HOUR	R. Q. (45 minute periods) (1)	(2)	(3)	REMARKS
1926										
March										
17	9:20 a.m.	6.84	3.14	2.18						
	3:15 p.m.	6.56	2.35	2.80	1.09	0.39				
	3:20	150 cc. blood serum and 20 gram dextrose and 200 clinical units insulin by stomach tube					?	1.06	0.71	
	6:15	9.60	1.48	6.50	3.20	0.49				
18	9:16 a.m.	14.86	5.64	2.65	0.99	0.37				
	9:20	150 cc. blood serum and 20 gram dextrose (no insulin) by stomach tube					0.80	0.82	0.88	Vomited small amount
	12:16 p.m.	9.04	1.16	7.70	3.01	0.39				
	9:36	9.70	3.80	2.55	1.00	0.41				
19	9:15 a.m.	3.06*	1.24	2.47						
	9:30–12:00	basal respiratory metabolism					0.77	0.72		
	12:15	0.0	0.62		0.0	0.20				
	12:20	150 cc. blood serum and 20 gram dextrose and insulin by stomach tube					0.80	0.57†		Dog died March 20 from overdose subcutaneous insulin
	3:20	1.21	0.60	2.0	0.40	0.20				

* Bladder urine sugar-free.

† Known leak in apparatus.

serum alone. In an experiment with dog V this order was reversed (see page 159). The amount of sugar excreted was again small as compared with the amount placed in the stomach. The dog vomited a small portion which could not be recovered, so that the exact figure for ingestion cannot be stated, but it could not have been less than 17 grams pure d-glucose. The retention for the three-hour period may be calculated as 17 − (9.04 −

3.00) = 11 grams, to say nothing of sugar which might have come from the serum proteins digested. The excretion of sugar for the subsequent period was the same as before the sugar and blood serum were given. The more even combustion indicated by the R.Q.'s 0.80, 0.82 and 0.88 certainly means that there was no shock-like alteration of the metabolism such as one gets from subcutaneous administration or from a sudden access of insulin from the alimentary tract.

TABLE 2

Dog II

DATE	TIME	TOTAL D	TOTAL N	D/N	D PER HOUR	N PER HOUR	R.Q. (45 minute periods) (1)	(2)	(3)	REMARKS
1926										
April										
12	9:10 a.m.	Dog fed small amount meat: placed in respiratory apparatus					0.69	0.71		
13	9:10	1.13	0.309	3.65						
	11:25	1.92	1.16	1.69	0.85	0.51				
	11:45	150 cc. blood serum and 20 gram dextrose and insulin by stomach tube					0.80	0.97	0.72	
	3:40 p.m.	1.20	0.69	1.74	0.28*	0.16				Dog vomited large amount
14	8:55 a.m.	2.47	0.78	3.2						
	9:05	150 cc. blood serum and 20 gram dextrose (no insulin) by stomach tube					0.75	0.72	0.72	
	12:15 p.m.	0.48†	0.11							Dog vomited

* 6.24 grams dextrose absorbed.

† 6.28 grams dextrose absorbed.

The next morning the urine found in the bladder was free of sugar and for the basal period, 9:30 to 12:00, no sugar was excreted. The basal R.Q.'s were those of a normal fasting dog. Insulin in blood serum at noon given with dextrose again raised the R.Q. to 0.80 the first period, but the next period was lost on account of a leak in the apparatus. When the urine became sugar free the rate of nitrogen excretion was diminished as it should be by the sparing effect of sugar oxidized.

This dog was killed accidentally by too large a dose of insulin subcutaneously the next day. Autopsy revealed no trace of pancreas.

Dog II. Female spaniel weight 18.2 kgm. pancreatectomy April 8, 1926. The dog was treated with insulin subcutaneously on the 9th and 10th. On the 11th and subsequently no insulin except by stomach tube. Dog retained no food during the course of the following experiment.

Insulin, 100 clinical units in 150 cc. blood serum, placed in the stomach with dextrose caused an increase in R.Q. from basal of 0.70, obtained the day before, to 0.80, first period and 0.97 second, followed by a return to 0.72 the third, a typical effect such as one obtains from subcutaneous injections. The absorption of insulin must have been almost as rapid as from the subcutaneous tissues. A control next day with blood serum and dextrose alone raised the R.Q. only to 0.75. The dog vomited and urinated in the respiration chamber both days but it was possible to recover all of the sugar and to calculate the amount of sugar available for absorption both days. Unaccounted for by vomitus or urine on the 13th were 6.24 grams and on the 14th 6.28 grams.

Because the dog vomited after administration of insulin there might be a suspicion that absorption of the hormone could have taken place from the mouth; but the vomiting did not occur until after the last R.Q. period recorded.

Dog III. Female mongrel; pancreatectomy by Doctor Angevine on May 18, 1926. Fed meat twice daily and given insulin in ox-blood serum + 5 per cent alcohol twice daily until May 23. May 25–28, no insulin. May 29 to June 3, 0.8 cc. U-20 "Iletin" given subcutaneously. June 4 and 5, no insulin. June 6, 0.8 cc. U-20 "Iletin." June 7, no insulin. Experiment started on 8th. (Table 3.)

The D:N ratio was 2.95 both before and during the basal metabolism period. The R.Q.'s however were not so low as those usually obtained in a totally depancreatized dog without insulin. It is possible there was a slight holdover effect of insulin given on the 6th.

After 40 clinical units of insulin were placed in the stomach with blood serum and dextrose the R.Q. rose the first period to 0.87 and there was a diminished excretion of sugar, in spite of the blood proteins ingested, which continued for the next 3-hour period. The excretion of sugar for the $3\frac{1}{3}$ hours which included the respiration experiment, without insulin and sugar, would have been about 2.0 grams. Actually found in the urine were 9.65 grams which would include at the least 7.65 grams (probably more) from the 10 grams (gross) or 9.29 grams (net) d-glucose ingested. Available for absorption was therefore at least 1.64 gram which would be more than sufficient without drawing any from the blood, to account for the rise of R.Q. noted. The absorption of insulin seems to have been slower than in the previous experiments; for the second period which usually shows the highest R.Q. recorded a return nearly to basal. Its absorption must have continued through the night, however, and have affected the

TABLE 3

Dog III

DATE	TIME	TOTAL D	TOTAL N	D/N	D PER HOUR	N PER HOUR	R. Q. (45 minute periods) (1)	(2)	(3)	BLOOD SUGAR	REMARKS
1926											
June											
8	9:10 a.m.	1.77	0.60	2.95							Dog fed meat only since June 3
	9:30–11:30	In respiratory apparatus					0.71	0.72			
	11:50	1.73	0.60	2.95	0.65	0.23					
	12:00 m.	170 cc. blood serum and 10 gram dextrose and 40 units insulin by stomach tube					0.87	0.73			
	3:10 p.m.	9.65	0.82		2.89	0.19					
	6:15	2.93	0.57		0.95	0.19					6:15 p.m. 200 grams Hamburg, bone ash, pancreatin
9	8:55 a.m.	1.65	1.52	1.07							
	9:00–11:00	In respiratory apparatus					0.79	0.87			
	11:30	1.23	0.47	2.61	0.48	0.18					
	11:45	160 cc. blood serum and 40 units insulin (no sugar) by stomach tube					?	0.97	0.92		
	3:15 p.m.	3.33	0.78	4.27	0.89	0.21					
	6:00	0.66	0.42		0.24	0.15					6:15 p.m. 200 grams Hamburg, bone ash, pancreatin
10	8:45 a.m.	0.27	0.23	1.16							
	9:00–11:00	In respiratory apparatus					0.77	0.71			
	11:40	0.61	0.53	1.15	0.21	0.18				0.355	
	11:50	50 cc. blood serum and 5 cc. 95 per cent alcohol and insulin by stomach tube					0.77	0.76	0.80		
	3:15 p.m.	1.53	0.66	2.32	0.43	0.19				0.337	

TABLE 3—*Concluded*

DATE	TIME	TOTAL D	TOTAL N	D/N	D PER HOUR	N PER HOUR	R. Q. (45 minute periods) (1)	(2)	(3)	BLOOD SUGAR	REMARKS
1926											
June 10	5:55	0.89	0.42	2.1	0.33	0.16					6:00 p.m. 300 grams Hamburg, bone ash, pancreatin
11	9:05 a.m.	0.0									
	9:30–11:30	In respiratory apparatus					0.74	0.84			
	11:40	0.0	0.61			0.24				0.306	
	11:55	50 cc. blood serum and 5 cc. 95 per cent alcohol and 60 units insulin by stomach tube					?	0.77	0.73		
	3:15 p.m.	0.0	0.61			0.17				0.305	
	5:55	0.0	0.61			0.23					
14	9:05 a.m.	0.0									300 grams Hamburg, bone ash, pancreatin
	9:15–11:00	In respiratory apparatus					0.73	0.77			
	11:05	0.0	0.33			0.17					
	11:06	100 cc. blood serum and 10 grams dextrose and 40 units insulin by stomach tube									6:00 p.m. 300 grams Hamburg, bone ash, pancreatin, NaCl, 10 grams cracker meal
	11:10–2:10	In respiratory apparatus					0.75	0.87	0.82		
	2:15	6.36	0.52			0.16					
16	9:15 a.m.	0.0									
	9:20–11:30	In respiratory apparatus					0.72	0.76			Same on 15th
	11:35	0.0	0.54			0.23					
	11:38	50 cc. blood serum and 20 grams dextrose and 40 units insulin								R.Q.	
	11:40–3:55	In respiratory apparatus						0.80	0.77	0.695	Same with 20 gram cracker meal on 16th
	3:55	12.3	0.85			0.20					

gluconeogenesis; for the D:N ratio in the night urine following a meal of meat in the evening was only 1.07. There was ample protection from the pancreatin fed with the meat. The R.Q.'s obtained in two basal periods were higher than those of a normal dog on a meat diet.

As not infrequently happens with diabetic dogs, even when not treated, the first morning period on the 9th (8:55 to 11:30 a.m.) showed a higher D:N ratio than the night urine, and when 160 cc. blood serum and 40 units insulin were given the D:N rose even higher, indicating digestion of the blood proteins and the early elimination of the sugar as compared with the nitrogen (8). The blood serum contained 2.05 grams N but of this only 0.03 gram per hour appeared up to $2\frac{1}{2}$ hours after administration. In the totally diabetic animal this amount of nitrogen would yield in a 12-hour period some 5.3 grams extra dextrose (calculated on a D:N of 2.61). There were found in the urine at the end of $3\frac{1}{2}$ hours 3.33 grams. At the prevailing rate of excretion previous to insulin there would have been 1.80 gram (0.48×3.75 hours) of dextrose from the endogenous metabolism, leaving 1.53 gram (3.33–1.80) for sugar having its source elsewhere. Hence there would remain for excretion in the following periods at least two-thirds of the extra sugar. As a matter of fact the post-insulin excretion was much less than the pre-insulin excretion. The high R.Q.'s obtained, 0.97 and 0.92, show what became of at least a part of the sugar.

The D:N ratio is of little help at this point for the excretion of nitrogen indicates scarcely any digestion of the blood proteins. The insulin itself would yield only a few milligrams of nitrogen. We must wait for further information regarding the effects of insulin injected directly into the portal circulation before we shall be in position to interpret fully such results as those just discussed.

One thing, however, is clear; namely, that if there was no considerable digestion of blood proteins there must have been an abundant store of glycogen somewhere, and *probably* the blood sugar would have shown an appreciable fall, had it been recorded. The next day this defect was remedied.

On the 10th basal R.Q.'s of 0.77 and 0.71 were obtained together with the low D:N of 1.15 in the night urine, indicating on both hands maintenance of the improved metabolism. Forty clinical units of insulin in blood serum, without sugar, but with a small amount of alcohol were placed in the stomach, and the dog immediately returned to the respiration chamber. The hourly excretion of sugar was doubled without affecting the nitrogen excretion materially; but the R.Q. did not show any distinct rise until the third period. The alcohol 2.5 grams of which would depress the R.Q. at the metabolic rate of this dog quite 15 points in 2 hours was doubtless responsible for the delay.[3] The blood sugar fell from 0.355 to 0.337 in the

[3] In the 2nd period where the R.Q. was 0.76 the CO_2 output was 3.014 L and the O_2 absorption 3.95 L per hour. The burning of 2.5 grams alcohol in 2 hours would

period which included these R.Q.'s. Without any exogenous source of sugar some decline would have taken place but with a doubling of the hourly excretion it is fairly certain that the blood proteins were undergoing digestion and yielding sugar, the nitrogen-excretion being temporarily delayed. The fall in blood sugar therefore indicates greater combustion (or storage) of sugar which in this case is masked in the R.Q.'s by the combustion of alcohol.

The urine found in the bladder on the morning of June 11 was sugar-free and continued free until the 14th (when dextrose was given) notwithstanding the feeding of 300 to 500 grams Hamburg steak with pancreatin and bone ash every day. The amount of insulin which held the dog in this condition was only 40 clinical units given in blood serum. On the 11th, as on the 10th, the insulin was given with a small amount of alcohol also; but on the 12th, 13th and 14th no alcohol was added. The blood sugar on the 11th did not change after insulin as it had done on the 10th. The general level was lower than on the 10th, but was higher than the threshold for a normal dog. It is possible that a change in the permeability of the kidney had taken place; but if so, it was only temporary, as regards the rate of excretion of the nitrogen, for in the next 3-hour period this excretion returned to the original level for the day and even at the lowest it was but slightly below the general level for the whole experimental period. Moreover a decrease in permeability of any consequence would have caused a rise in blood sugar. Work in this laboratory (9) has shown that in the phlorhizinized dog the effect of insulin injected directly into the renal artery is to restore towards normal the increased permeability of the kidney to dextrose caused by the drug and to increase the permeability to nitrogen. If such an effect were present in the experiment before us it would explain the absence of a fall in the blood sugar but would leave us perplexed regarding the nitrogen. The behavior of the R.Q.'s after insulin is obscured again by the combustion of alcohol. Indeed, it is possible that the renal injury, if there was one, was produced by the alcohol which was given to take advantage of any possible stimulation to the absorption as reported by Winter (9). There was no such stimulation visible at any time.

The R.Q.'s just before insulin on the 14th confirm the state of metabolism indicated by the absence of sugar from the urine; namely, such as would be expected in a normal dog on a meat diet. Blood sugar, however, on the 15th was still much above the normal level. Could it be that insulin entering the portal circulation and regulated in its action by the liver, to

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produce 1.8 L CO_2 per hour and absorb 1.2 L O_2 per hour, a difference of 0.6 L. Adding this 0.6 L as CO_2 to 3.014 L, giving 3.614, would produce an R.Q. of 0.91 instead of 0.76.

which it is normally conveyed by the pancreatic veins, affects chiefly the respiratory metabolism and much more slowly, if at all, the general level of the blood sugar? Hoskins and Snyder (11) in work recently published from the department of Anatomy of this institution have shown that insulin injected into the peritoneal cavity of well-fed rabbits does not lower the blood sugar, although it does do so in fasting rabbits. The insulin must be absorbed directly into the portal circulation; but with carbohydrate in abundance traversing the same pathway it does not get through to the general circulation so as to cause the usual explosive increase in combustion in the muscles. Possibly an explosive increase in combustion takes place in the liver itself without interfering with the supply of sugar to the general circulation. However this may be, to understand the experiment before us, we have only to suppose that with sugar (or glycogen) available in the liver, a moderate dose of insulin is completely employed in stimulating the disposal of carbohydrate locally. With a larger dose, however, or with a second dose, as we shall see presently, some of it gets through to the general circulation and influences metabolism more widely and thus affects the general level of the blood sugar. The liver absorbs the shock-like effect on the blood sugar. This has been confirmed recently in this laboratory by direct injection into the portal vein of dogs under amytal anaesthesia.

Meantime we may note that the urine contained sugar on the 14th when 10 grams dextrose were given with the insulin and again on the 16th when 20 grams dextrose were given. The R.Q.'s on these two days show effects of the same general character—a basal level somewhat lower than that of a normal dog on meat diet, and a stimulating effect on combustion which is most marked in the second period.

Effects of insulin by alimentary tract on blood sugar. Experiments designed to exhibit effects upon the respiratory metabolism are often not suited to show effects on blood sugar. For example, when dextrose was given with the insulin by stomach tube there would be for several hours a rise in blood sugar because of more rapid absorption than combustion and excretion combined, and the exact balance of these factors could never be predicted with any certainty because of the variations in absorption of the insulin itself. For this reason a separate day, June 15, was selected for exhibiting the effects on blood sugar. Dog IV which had been depancreatized on June 2 and treated with subcutaneous insulin until June 11 was fed in the same way as dog III on June 12, 13 and 14 and given 40 clinical units of insulin in blood serum daily for these three days. This dog later proved to be pregnant and for this reason had a much lower blood sugar. The results of two doses of 40 clinical units each on both dogs are given in table 4.

With dog III there was no decrease in blood sugar following the first dose but rather an increase due no doubt to slow digestion and absorption from the alimentary tract.

With dog IV there was a perceptible drop at the end of 3 hours followed by a rise at 4 hours. With both dogs there was a substantial drop, 66 and 32 mgm. respectively, following the second dose at two hours. The alimentary tract at this time was empty of food, giving better opportunity for absorption of the insulin.

The tests were repeated in a different way on both dogs on July 2. The results are given in table 5.

TABLE 4

Dogs III and IV—Effect of insulin by stomach tube on blood sugar

TIME	DOG III	DOG IV
9:35 a.m.	0.403	0.134
9:36	Given 50 cc. blood serum and 40 clinical units insulin	
11:35	0.412	0.137
12:30 p.m.	0.420	0.128
1:35	0.426	0.137
2:40	0.420	0.132
3:05	Given 50 cc. blood serum and 40 clinical units insulin	
5:00	0.354	0.100

TABLE 5

Dogs III and IV—Effects of repeated small doses of insulin in blood serum by stomach tube

TIME	DOG III	TIME	DOG IV
9:55 a.m.	0.311	9:45 a.m.	0.254
10:00	50 cc. blood serum + 20 clinical units	9:50	Same as dog III
11:50	0.306	11:45	0.265
11:55	50 cc. blood serum + 20 clinical units	11:51	Same as dog III
1:50 p.m.	0.305	1:45 p.m.	0.243
1:55	50 cc. blood serum + 20 clinical units	1:51	Same as dog III
3:50	0.297	3:45	0.230
3:55	50 cc. blood serum + 20 clinical units	3:51	Same as dog III (vomited)

Dog IV this time showed the better effect but in neither case was the effect so great as when the same number of units were administered in two doses. In fact it is difficult to be certain in either case that the slight fall in blood sugar was not merely the decline with diminished absorption of foods as the alimentary tract became empty.

SURVIVAL OF DEPANCREATIZED DOGS ON INSULIN ABSORBED FROM ALIMENTARY TRACT. Dogs III and IV were kept alive on insulin in blood serum

for several months. Their protocols for the period not already covered by the preceding experiments follow.

Dog III. June 18–28, 100 cc. blood serum + 40 clinical units 9:00 a.m. and 4:00 p.m. Food 200 grams ground beefsteak, 20 grams cracker meal, bone ash and pancreatin 10:00 a.m. and 4:30 p.m.

June 29, weight 8.8 kgm.

June 28–July 7, 100 cc. blood serum + 40 clinical units a.m. and p.m. Food 200 grams ground beefsteak, 10 grams lard, bone ash, NaCl and pancreatin (about 1 gram) a.m. and p.m.

July 7, weight 7.9 kgm.

July 13 to July 21. Added 10 per cent alcohol to blood serum. Food as before. Insulin was increased gradually to 200 clinical units daily.

July 21, weight 6.8 kgm. *No urine all day!*

July 22 to August 7. No alcohol in blood serum. Food same as before.

August 4, weight 6.1 kgm.

August 7. Dog found dead. Weight 5.8 kgm. Autopsy revealed a granulomatous mass at site of pancreas about $\frac{1}{10}$ size of pancreas removed. Section showed no pancreatic tissue.[4]

Dog IV. As stated above (page 156) this dog had been depancreatized on June 2 and was kept on subcutaneous insulin until June 12, when insulin in blood serum by stomach tube was begun.

June 12–14. One dose 70 cc. blood serum + 40 clinical units. Food once daily, 300 grams ground beefsteak + 10 grams cracker meal + bone ash, salt and pancreatin (about 1 gram).

June 16–17. Same except food contained 20 grams cracker meal.

June 17, weight 10.7 kgm.

June 18–27. 100 cc. blood serum + 40 clinical units a.m. and p.m. Food 200 grams ground beefsteak, 20 grams crackermeal, bone ash, salt and pancreatin (about 2 grams) twice daily.

June 27, weight 11.05 kgm.

June 28–July 2. Insulin, same food, 100 grams ground beefsteak + 30 grams lard + 5 grams cane sugar + bone ash, salt and pancreatin (2 gm.) twice daily.

July 2–5. Three doses, 20 clinical units, insulin in 100 cc. blood serum at 9:00, 1:00 and 3:00. Food, 200 grams ground beefsteak, 10 grams lard, bone ash, salt and pancreatin (about 1 gm.) twice daily at 11:00 a.m. and 5:00 p,m.

July 5, weight 11.1 kgm.

July 13–23. Added 10 per cent alcohol to blood serum. Insulin increased to 120 clinical units daily: food as from July 2.

July 23. Urine was sugar free.

July 16–July 23, weight constant 10.6 kgm.

July 29. Four pups born, one dead.

August 2. All pups dead.

August 4, weight 8.3 kgm.

August 7, weight 8.5 kgm.

August 8–September 6. Insulin gradually increased to 360 clinical units daily, food remaining the same.

September 7, weight 6.9 kgm. Dog during all this time excreted only a small amount of sugar; barely positive qualitative test with Benedict's reagent. Urine volume about 500 cc. daily.

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[4] The authors are indebted to Dr. Geo. A. Corner, Professor of Anatomy, whose technicians prepared these sections.

September 7–October 4. Insulin continued at 3 doses of 120 units each in blood serum; food same as before.

October 4, weight 5.9 kgm. Dog killed. Autopsy revealed a small piece of pancreas about the size of a navy bean at site of the small duct. Evidently regenerated from the small duct; section proved presence of islet cells.

The failure to maintain weight in these two dogs was found by subsequent experience to be due to an inadequate amount of pancreatin for artificial digestion and to failure of appetite. Dog III upon only a few days' treatment with subcutaneous insulin, all the rest of the time upon insulin in blood serum by stomach tube survived 81 days. Dog IV, pregnant at time of operation, likewise treated with subcutaneous insulin for only 10 days, all the rest of the time upon insulin in blood serum by stomach tube, survived 125 days, 67 of them after birth of the pups. She certainly would have lived some weeks longer. The small bit of pancreas regenerated in dog IV certainly not more than $\frac{1}{50}$th the size of the original pancreas, could not have figured materially in this result, since F. M. Allen many years ago demonstrated that $\frac{1}{5}$th of the mass is necessary to maintain a dog sugar-free. The slight gain in weight made by dog IV from June 17 to July 5 probably is related to the pregnancy for, as is well known, depancreatized dogs will survive the period of pregnancy without insulin. Quite possibly the death of the pups so soon after birth is to be ascribed to the alcohol used from July 13 to parturition.

In order to see how long a dog would survive after complete pancreatectomy upon insulin in blood serum by stomach tube, but without artificial digestion the following experiment was made upon dog V.

Dog V. Female, black and white hound weighing 7.0 kgm.; pancreatectomy September 13, 1926. Given water only first day and small amount meat. Meat increased to 200 grams 2nd day. On 17th began experimental work. No insulin in any form until 18th.

September 17. Weight, 6.3 kgm. Food every day ground beefsteak, lard and bone ash; no pancreatin.
9:10–12:20 Basal R.Q.'s 45 minute periods, 0.68, 0.73.
12:35 Gave 20 grams Merck's dextrose.
1:00–4:00 R.Q.'s 45 minute periods, 0.78, 0.69, 0.69.

September 18. 9:25 a.m. Urine D:N 2.40
9:30 70 cc. blood serum + 20 grams dextrose.
9:45–12:20 R.Q.'s 0.68, 0.79, 0.79.
12:35 70 cc. blood serum + 20 grams dextrose + 100 clinical units insulin.
12:50–3:20 R.Q.'s 0.87, 1.15, 0.74.

September 19. 9:45 Bladder urine D:N 2.20

21. Weight 5.9 kgm. Urine sugar-free, no insulin since September 18.

22. 70 cc. blood serum + 20 grams dextrose + 100 clinical units insulin.

27. Weight, 5.5 kgm. No insulin since 22nd, −20 clinical units 3:00 p.m.

September 27. Food changed to 100 grams meat, 10 grams lard, bone ash, twice daily at 11:00 and 4:00 o'clock.
28. Urine sugar-free.
29. Weight 5.3 kgm.
30. Urine nearly sugar-free.
October 5. Weight, 4.9 kgm. No insulin since September 28, 20 clinical units.
6. 2 doses, 20 clinical units each.
7. Weight, 4.9 kgm. Urine nearly sugar-free.
Food increased to 150 grams meat + 10 grams lard + bone ash twice daily.
9. 3 doses 20 clinical units each.
11. Weight 4.2 kgm. 4 doses 20 clinical units each. More sugar in urine.
13. Weight, 4.05 kgm. Food increased to 200 grams meat, 10 grams lard, 5 grams cracker meal, bone ash, pancreatin added for first time, twice daily.
15. Weight, 4.1 kgm. Does not eat all of food. 20 clinical units insulin. Pancreatin replaced by 50 grams fresh pig's pancreas.
16. Sugar in urine increased.
18. Weight, 3.9 kgm. No insulin since 15th. Pig pancreas continued.
2:30 gave 100 clinical units in 70 cc. blood serum + 20 grams dextrose.
2:45–5:00. R.Q.'s 45 minute periods 0.68, 0.74, 0.71.
Evidently fresh pancreas destroys insulin.
19. Dog used for x-ray study. Good subject because of emaciation. Given barium in milk at 7:00 a.m. Regular feeding p.m. No fresh pancreas fed.
20. Weight, 3.9 kgm. Given 100 cc. milk + 60 clinical units insulin at 1:00 and 3:00 o'clock in addition to regular feedings. (No artificial digestion.)
22. Weight, 3.65 kgm. Sugar greatly increased in urine, 11–1:30 R.Q.'s 0.75, 0.74.
1:45– given 100 clinical units in blood serum + 20 grams dextrose.
2:00–4:45 p.m. R.Q.'s 0.63, 0.99, 0.87.
September 23. Dog refused food. Too weak to stand. Killed at 2:00 p.m.
Autopsy: Site of pancreas carefully explored and every suspicious bit of tissue preserved and sectioned.[5] No pancreatic tissue found.

Dog survived 41 days from operation and would have lived several days longer. No insulin was given at any time except by stomach tube.

It is worthy of note that on September 18 when blood serum and dextrose were given without insulin there was a distinct rise in the R.Q.'s the second and third periods; but not nearly so great as when, in the afternoon, insulin was added (see page 149).

This dog maintained weight for three short periods, October 5 to 7, 13 to 15, when pancreatin was added to the food, and 18 to 20, when the dog had received a large dose of $BaCO_3$ in milk for an x-ray study of the intestine. There is no possible explanation for the first success, except that the dog had received one dose of insulin on the 5th and two on the 6th and was practically sugar-free for the three days. In the second period food

[5] See footnote, page 158.

was eaten in larger quantity and pancreatin was used each day, but the appetite failed on the second day. No insulin had been given since the four small doses on the 11th. But the urine contained large amounts of sugar. The last period of stationary weight doubtless was due to barium carbonate retained. It seems indicated that the use of artificial digestion, some means of maintaining appetite and intermittent use of insulin are the conditions which might be successful in maintaining weight even though the diabetic condition were not fully controlled.

At the time of writing another dog is still alive 13 weeks from the time of operation and at times has been made to gain weight at the rate of 1 kgm. a week in spite of an intense glycosuria. Details of this experiment will be reserved for a later publication: but it may be stated here that the use of liver and liver extract for maintenance of appetite and the use of as much as 30 grams pancreatin daily were found necessary for this large gain. Insulin in blood serum by stomach tube was given two and three times daily. No insulin at any time by subcutaneous injection.

SUMMARY AND CONCLUSIONS

1. Observations on a series of depancreatized dogs prove beyond a doubt that insulin protected by blood serum from the proteolytic destruction of the digestive enzymes can be absorbed in significant amounts from the alimentary tract.

2. In some instances the respiratory quotient was raised quite as high as following subcutaneous injection.

3. In several instances the urine was made entirely sugar-free although the dog was on full diet containing meat.

4. The effect on blood sugar is not so sudden or so great as when insulin is given subcutaneously. The liver seems to protect the body against the shock-like effects of a sudden access of insulin to the general circulation. Hypoglycemic shock has never been seen following alimentary insulin even in huge doses.

5. Four dogs have been kept alive 81, 125, 41 and 91 days respectively after complete pancreatectomy and while on full diets. The dog which survived longest was pregnant, but lived 67 days after birth of the pups.

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ON THE "CALORIGENIC ACTION" OF EPINEPHRIN

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That property of protein, when ingested, of causing an increased heat output which is independent of the external activity of the organism, was named "specific dynamic action" by Rubner (1902). Among others this investigator also demonstrated that the increased metabolism observed could not be due to the intestinal activity involved in the digestion and absorption of the foodstuff. He ascribed the phenomenon rather to the appearance of heat produced by the intermediary metabolism of the protein, over and above the level of energy production maintained by actual cell metabolism.

Lusk (1921), however, from the results of numerous experiments, concluded that the cause of the specific dynamic action of protein was "a chemical stimulation of the cells, causing them to metabolize more material." In this he agrees with the earlier views of Voit (1881), and has been supported by some later work, the most recent perhaps being the demonstration by Rapport and Katz (1927) of an apparently similar effect of glycine on the metabolism of isolated perfused muscle tissue.

Various workers have also shown an apparently similar effect of epinephrin in increasing the metabolic process. Thus Boothby and Sandiford (1920) found that among other effects, the subcutaneous injection of 0.5 cc. of $\frac{1}{1000}$ adrenalin chloride solution in man invariably caused an increase in the metabolic rate and, in most cases, a rise in the respiratory quotient. It was suggested that "the similarity of the metabolic rate curve following the injection of adrenalin, to that found by Lusk from a carbohydrate plethora, naturally directs attention to the possibility that the increased heat production is due to an excess of carbohydrate metabolites. In addition there may be, however, a direct chemical stimulation of cellular combustion." Later (1923), in the light of results obtained from experiments on dogs, these workers concluded that since the increase in heat production was too great to be entirely accounted for by the slight increase in blood sugar concentration, the effect of epinephrin was due rather to a direct stimulation of cell metabolism. In this paper also the authors, at the request of Lusk, substituted the term "calorigenic action" for "specific dynamic action" in order to maintain the sense of the latter term as

TABLE 1

Effect of epinephrin on the respiratory exchange in normal dogs

DOG NUMBER	TIME	O_2 INTAKE	R.Q.	REMARKS
		cc. per kilo per hour		
1				Fasted 48 hours. Weight, 16.0 kilo
	10:30 a.m.	369	0.703	Circuit closed at 9:30 a.m.
	11:30 a.m.	343	0.724	
	11:35 a.m.	*Epinephrin injected*		Circuit closed at 11:55 a.m.
	12:55 p.m.	487	0.777	
	1:55 p.m.	580	0.825	
	2:55 p.m.	513	0.856	
	3:55 p.m.	478	0.848	Average cabinet temperature, 23.6°C.
2*				Fasted 48 hours. Weight, 12.4 kilo
	11:25 a.m.	646	0.706	Circuit closed at 10:25 a.m.
	11:50 a.m.	*Epinephrin injected*		Circuit closed at 12:10 p.m.
	1:10 p.m.	750	0.755	
	2:10 p.m.	877	0.734	
	3:10 p.m.	697	0.751	
	4:10 p.m.	725	0.711	Average cabinet temperature, 23.5°C.
11				Fasted 24 hours. Weight, 6.0 kilo
				Blood sugar at 11:30 a.m., 0.120 per cent
				Injected 3 grams glucose intravenously
				Injected 3 grams glucose subcutaneously
				Blood sugar at 11.35 a.m., 0.234 per cent
	1:04 p.m.	736	0.793	Circuit closed at 12:04 p.m.
	2:04 p.m.	506	0.747	Blood sugar at 2:15 p.m., 0.119 per cent
	2:16 p.m.	*Epinephrin injected*		Circuit closed at 2:45 p.m.
	3:45 p.m.	1001	0.801	
	4:45 p.m.	995	0.821	
	5:45 p.m.	907	0.764	Average cabinet temperature, 24.4°C.
13				Fasted 24 hours. Weight, 5.0 kilo
				Injected 10 grams glucose intravenously
				Injected 9 grams glucose subcutaneously
	11:12 a.m.	719	0.925	Circuit closed at 10:12 a.m.
	12:12 p.m.	597	0.868	Blood sugar at 12:17 p.m., 0.140 per cent
	12:20 p.m.	*Epinephrin injected*		Circuit closed at 12:40 p.m.
	1:32 p.m.	842	0.906	
	2:07 p.m.	1020	0.920	
	3:02 p.m.	924	0.873	
	4:07 p.m.	965	0.808	Average cabinet temperature 23.6°C.

* This dog later found to be pregnant.

TABLE 2

Effect of epinephrin and glucose on the respiratory exchange in eviscerated dogs

DOG NUMBER	TIME	O_2 INTAKE	R.Q.	REMARKS
		cc. per kilo per hour		
3				Fasted 24 hours. Weight, 9.5 kilo
				Injected 10 grams glucose intraperitoneally
				Injected 10 grams glucose subcutaneously
	1:00 p.m.	525	0.895	Circuit closed 12:00 noon
	2:00 p.m.	552	0.845	
	2:04 p.m.	*Epinephrin injected*		Circuit closed at 2:30 p.m.
	3:30 p.m.	460	0.856	
	4:30 p.m.	422	0.854	
	5:20 p.m.	442	0.827	Average cabinet temperature 25.3°C.
4				Fasted 24 hours. Weight, 6.0 kilo
				Injected 20 grams glucose intraperitoneally
				Injected 10 grams glucose subcutaneously
	12:40 p.m.	291	0.983	Circuit closed at 11:40 a.m.
	1:40 p.m.	281	0.979	
	1:43 p.m.	*Epinephrin injected*		Circuit closed at 2:05 p.m.
	3:05 p.m.	232	1.03	
	4:05 p.m.	209	1.08	
	5:05 p.m.	200	1.08	
	6:05 p.m.	189	1.10	Average cabinet temperature 23.8°C.
6				Fasted 24 hours. Weight, 5.5 kilo
				Injected 20 grams glucose intraperitoneally
				Injected 10 grams glucose subcutaneously
				Injected 3 grams glucose intravenously
	11:55 a.m.	853	0.942	Circuit closed at 10:55 a.m.
	12:55 p.m.	465	0.944	
	1:00 p.m.	*Epinephrin injected*		Circuit closed at 1:17 p.m.
	2:17 p.m.	320	0.930	
	3:17 p.m.	315	0.965	Average cabinet temperature 23.8°C.
9				Fasted 24 hours. Weight, 8.5 kilo
				Injected 20 grams glucose intraperitoneally
				Injected 10 grams glucose subcutaneously
				Injected 5 grams glucose intravenously

TABLE 2—*Concluded*

DOG NUMBER	TIME	O_2 INTAKE	R.Q.	REMARKS
		cc. per kilo per hour		
	12:06 p.m.	550	0.984	Circuit closed at 11:06 a.m.
	1:06 p.m.	446	0.934	
	2:06 p.m.	509	0.956	
	2:19 p.m.	*Epinephrin injected*		Circuit closed at 2:32 p.m.
	3:32 p.m.	456	0.909	
	4:32 p.m.	455	0.885	
	5:32 p.m.	374	0.952	Average cabinet temperature 24.6°C.
10				Fasted 24 hours. Weight, 3.0 kilo *Injected 3 grams glucose intravenously*
	12:22 p.m.	725	1.00	Circuit closed at 11:22 a.m.
	1:22 p.m.	527	0.931	
	2:22 p.m.	331	0.946	
	2:55 p.m.	286	0.830	
	3:06 p.m.			*Injected 5 grams glucose intravenously* Circuit closed 3:25 p.m.
	4:25 p.m.	392	0.937	
	5:10 p.m.	289	0.978	Average cabinet temperature 21.6°C.
17				Fasted 24 hours. Weight, 6.5 kilo *Injected 20 grams glucose intraperitoneally* *Injected 10 grams glucose subcutaneously* *Injected 3 grams glucose intravenously*
	11:40 a.m.	272	0.897	Circuit closed 11:10 a.m.
	12:10 p.m.	259	0.966	
	12:12 p.m.	*Epinephrin injected**		Circuit closed 12:30 p.m.
	1:30 p.m.	223	0.871	
	2:30 p.m.	124	0.863	
	3:00 p.m.	117	0.861	Average cabinet temperature 23.1°C.

* Approximately four times the usual dose.

originated by Rubner, to indicate the increased metabolism following the ingestion of foodstuffs.

METHODS. The present investigation concerns the effect produced on the respiratory metabolism in normal, eviscerated, hepatectomized and depancreatized dogs, by the subcutaneous injection of 0.1 cc. of $\frac{1}{1000}$ adrenalin chloride (Parke, Davis & Co.) per kilogram body weight. A carefully controlled respiratory cabinet of the Benedict type was used. Any movements made by the animal while in the cabinet were graphically recorded.

The eviscerated preparations were obtained by a slight modification of Mann's (1925) method of total intra-abdomenectomy. Hepatectomy was

performed by a method described in a publication now in press. This method, like that originated by Mann (1921) permits of complete removal of the liver, but without the necessity of establishing an Eck fistula. Complete pancreatectomy was performed with the usual precautions. In every case the diabetic animals were deprived of all food and insulin for the forty-eight hours preceding the day on which the respiratory observations were made.

RESULTS. The protocols are grouped according to the type of animal preparation used.

DISCUSSION. The results show that the subcutaneous injection of 0.1 cc. of $\frac{1}{1000}$ solution of adrenalin chloride per kilogram body weight causes:

a. A rise in both O_2 consumption and R.Q. in normal dogs.

TABLE 3

Control eviscerated dog, given initial dose of glucose, but no epinephrin

DOG NUMBER	TIME	O_2 INTAKE	R.Q.	REMARKS
		cc. per kilo per hour		
18				Fasted 24 hours. Weight, 10.0 kilo
				Injected 15 grams glucose intraperitoneally
				Injected 15 grams glucose subcutaneously
				Injected 10 grams glucose intravenously
	12:15 p.m.	514	0.931	Circuit closed at 11:15 a.m.
	1:15 p.m.	296	0.869	
	2:15 p.m.	302	0.903	
	3:15 p.m.	248	0.877	
	4:15 p.m.	269	0.925	
	5:15 p.m.	242	0.879	
	6:15 p.m.	237	0.931	Average cabinet temperature 23.2°C.

b. No alteration in either the O_2 consumption or the R.Q. in eviscerated or hepatectomized dogs.

c. A rise in the O_2 consumption but no change in the R.Q., in depancreatized dogs. With larger doses, as noted in the protocols, similar results were obtained.

In order that the eviscerated and hepatectomized animals might live a sufficient length of time, it was necessary to give them rather large doses of glucose before placing them in the cabinet. The resulting rise of the R.Q. almost to unity may have masked any further rise subsequent to injection of epinephrin. The glucose administration, however, could not have interfered with the calorigenic influence of the epinephrin, as is evidenced by the control experiments with glucose and epinephrin on normal

dogs 11 and 13. Furthermore, for the short period that the eviscerated animal receiving no glucose survives, the oxygen consumption is quite comparable with that of preparations given glucose, once the relatively short initial effect of the carbohydrate plethora has disappeared.

That it was not impossible, or even difficult, to raise the oxygen consumption and R.Q. of the eviscerated preparations used, is shown by the

TABLE 4

Effect of epinephrin on the respiratory exchange in hepatectomized dogs

DOG NUMBER	TIME	O_2 INTAKE	R.Q.	REMARKS
		cc. per kilo per hour		
12				Fasted 24 hours. Weight, 7.7 kilo
				Injected 10 grams glucose intraperitoneally
				Injected 20 grams glucose subcutaneously
				Injected 10 grams glucose intravenously
	12:03 p.m.	275	1.04	Circuit closed at 11:03 a.m.
	1:03 p.m.	260	1.06	
	2:03 p.m.	240	1.13	Blood sugar at 2:20 p.m., 0.355 per cent
	2:22 p.m.	*Epinephrin injected*		Circuit closed 2:35 p.m.
	3:35 p.m.	142	1.12	Average cabinet temperature 22.3°C.
14				Fasted 24 hours. Weight, 10.0 kilo
				Injected 15 grams glucose intraperitoneally
				Injected 20 grams glucose subcutaneously
				Injected 5 grams glucose intravenously
	12:28 p.m.	452	0.930	Circuit closed at 11:28 a.m.
	1:28 p.m.	384	1.00	
	1:35 p.m.	*Epinephrin injected*		Circuit closed at 1:50 p.m.
	2:50 p.m.	323	0.984	
	3:20 p.m.	321	0.943	
	4:20 p.m.	299	0.902	*Injected 10 grams glucose intravenously 4:33 p.m.*
	5:54 p.m.	320	0.952	Circuit closed at 4:54 p.m.
	6:54 p.m.	315	0.932	Average cabinet temperature 23.1°C.

marked effects produced by the injection of glucose in dog 10, after a stay in the cabinet of over $3\frac{1}{2}$ hours. Nor can the lack of available muscle glycogen account for the negative results with epinephrin in eviscerated animals, as is shown by table 6, where the muscle glycogen is still considerable 2–$2\frac{1}{2}$ hours after evisceration and 1 to 2 hours after epinephrin administration. Furthermore, the failure to demonstrate any rise in the blood lactic acid

TABLE 5

Effect of epinephrin on the respiratory exchange in depancreatized dogs

DOG NUMBER	TIME	O_2 INTAKE	R.Q.	REMARKS
		cc. per kilo per hour		
7				Food and insulin withheld for 48 hours
	11:45 a.m.	546	0.720	Weight, 9.0 kilo Circuit closed 10:45 a.m.
	12:45 p.m.	581	0.703	
	12:52 p.m.	*Epinephrin injected*		Circuit closed 1:10 p.m.
	2:10 p.m.	885	0.710	
	3:13 p.m.			
	4:13 p.m.	830	0.701	
	5:13 p.m.	804	0.700	Average cabinet temperature 24.0°C.
8(a)				Food and insulin withheld for 48 hours
	9:30 a.m.	913	0.669	Weight, 6.0 kilo. Circuit closed 8:30 a.m.
	10:30 a.m.	835	0.703	Blood sugar at 10:40 a.m., 0.309 per cent
	10:45 a.m.	*Epinephrin injected*		Circuit closed at 11:02 a.m.
	12:02 p.m.	930	0.688	
	1:02 p.m.	950	0.692	
	2:02 p.m.	937	0.687	Blood sugar at 2:08 p.m., 0.327 per cent *Injected 3 grams glucose intravenously 2:10 p.m.*
	3:35 p.m.	914	0.690	Circuit closed 2:35 p.m.
	4:35 p.m.	986	0.698	
	5:35 p.m.	907	0.694	Blood sugar at 5:40 p.m., 0.308 per cent Average cabinet temperature 23.9°C.
8(b)				Food and insulin withheld for 48 hours
	9:35 a.m.	685	0.718	Weight, 5.5 kilo. Circuit closed 8:35 a.m.
	10:35 a.m.	714	0.706	*Injected 5 grams glucose intravenously, 10:50 a.m.* Circuit closed 11:10 a.m.
	12:10 a.m.	761	0.697	
	1:10 p.m.	748	0.727	
	2:10 p.m.	698	0.713	
	2:25 p.m.	*Epinephrin injected**		Circuit closed at 2:45 p.m.
	3:35 p.m.	908	0.713	
	4:45 p.m.	945	0.722	
	5:45 p.m.	1017	0.710	Average cabinet temperature 22.3°C.

* Approximately twice the usual dose.

in these animals, lends support to the view that there has been no increased breakdown of muscle glycogen following the injection of epinephrin.

Since the liver is the sole source of the glucose of the blood (cf. Bollman, Mann and Magath, 1925; Soskin, 1927) it might be assumed that the calorigenic effect of epinephrin in the intact animal is due to a carbohydrate plethora. As a matter of fact the injection of glucose in liverless animals does cause the O_2 consumption to increase, as the results of this research and that of Markowitz (in press) demonstrate. This, however, cannot be the important factor concerned since, when such a plethora is already existent, as in pancreatic diabetes or after raising the blood sugar by in-

TABLE 6*

Effect of epinephrin on the blood sugar, blood lactic acid and muscle glycogen in eviscerated dogs

DOG NUMBER	TIME	BLOOD SUGAR	BLOOD LACTIC ACID	REMARKS
		per cent	*per cent*	
1				Fasted 12 hours. Weight, 15.0 kilo
				Evisceration completed 2:30 p.m.
	2:45 p.m.	0.124	0.076	
	3:15 p.m.	0.097	0.030	
	3:17 p.m.	*1.5 cc.* $\frac{1}{10000}$ *epinephrin injected subcutaneously*		
	3:30 p.m.	0.091	0.018	
	4:00 p.m.	0.067	0.023	
	4:30 p.m.	0.054	0.012	Muscle glycogen: hind leg, 0.88 per cent, front leg, 1.16 per cent
11				Fasted 12 hours. Weight, 12.0 kilo
				Evisceration completed 2:00 p.m
	2:05 p m.	0.105	0.006	
	2:30 p.m.	0.085	0.008	
	2:32 p.m.	*1.0 cc.* $\frac{1}{10000}$ *epinephrin injected subcutaneously*		
	3:00 p.m.	0.073	0.007	
	3:30 p.m.	0.054	0.008	
	4:00 p.m.	0.051	0.008	
	4:20 p.m.	0.042		
	4:30 p.m.	0.042	0.008	Muscle glycogen; hind leg, 0.45 per cent; front leg, 0.91 per cent

* In collaboration with I. L. Chaikoff and J. J. Weber.

jections of glucose, epinephrin still displays its calorigenic action. Moreover, the fact that epinephrin produces its calorigenic action in the totally depancreatized animal and fails to do so in the hepatectomized dog, where the pancreas is intact, indicates that insulin can have no direct relationship to this function of epinephrin.

The present results, however, seem incompatible with the belief that epinephrin increases metabolism by direct stimulation of the tissue cells. Indeed, its failure to raise the oxygen consumption in liverless dogs must

be taken as direct evidence that such is not the case. This conclusion is supported by the work of Caskey (1927) who, by using the thermocouple to make direct observations on the temperature of the skeletal muscles, found that removal of the liver from the circulation prevented the usual increase in muscle temperature which follows the administration of epinephrin to normal animals. The liver must therefore be primarily responsible for the calorigenic effect of epinephrin.

CONCLUSIONS

1. Epinephrin causes a marked rise in the oxygen consumption and a significant increase in the R.Q. of normal dogs.

2. In eviscerated or hepatectomized dogs, epinephrin does not prevent the usual decline of the oxygen intake, or show any significant influence on the R.Q.

3. In depancreatized dogs, epinephrin causes a definite rise in the oxygen intake, but no significant change in the R.Q.

4. The carbohydrate plethora, caused by epinephrin in the normal dog, is not the essential cause of the increased metabolism which follows its administration.

5. Insulin bears no direct relationship to the calorigenic action of epinephrin.

6. The calorigenic action of epinephrin does not depend on direct stimulation of tissue cells, the presence of the liver being necessary for such an effect.

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RETENTION OF INTRAVENOUSLY INJECTED FLUID IN FASTING ANHYDREMIA AND PHLORHIZIN HYDREMIA

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The problem of maintaining adequate blood volume has received much attention from the viewpoint of the relative avidity of the blood for fluid. This is evidenced by the advances which have been made in the treatment of shock, by increasing the osmotic pressure of the circulating blood with hypertonic injections of acacia, dextrose, etc. To explain unsatisfactory results in the use of these methods, blame has been attached to increased permeability of the capillaries, while little if any attention has been paid to the condition of the body tissues in general.

So far as the present writers are aware, no one appears to have inquired to what extent, if any, the tissues can be induced to exert a protecting influence upon the circulation by refusal to deprive it of fluid.

The original point of attack in the present work was the question as to how a single organ like the liver, which Lamson and Roca's (1921) investigations have shown can serve as an important water reserve, could be influenced with respect to its avidity for fluid. Since the work of Rosenfeld (1902–3) it has been known that carbohydrate and fat storage in this organ were more or less mutually inhibitory. It would be inferred that the glycogen-rich, normal liver would store fluid more readily than the glycogen-poor, fatty liver.

We therefore produced a condition of fatty liver by means of phlorhizin injections, which are known to remove glycogen efficiently, as well as to transport fat from tissues to liver (Wertheimer, 1926). The accepted routine for phlorhizin poisoning involves fasting for several days; but when controls with fasting, non-phlorhizinized animals were made, we were surprised to find an equal change in the degree of retention of fluid in the circulation. Whatever was accomplished in this direction may be therefore attributed to the fasting alone; but a difference exists in the fact that retention after fasting starts from an anhydremic level of blood concentration,[2]—retention after phlorhizin from a level approaching hydremia.

[1] The investigations herein reported were assisted by a grant from the Ella Sachs Plotz Foundation.

[2] Morgulis and Edwards have demonstrated fasting anhydremia by the blood solids method. This Journal, 1924, lxviii, 497.

PROCEDURES. The experiments were performed upon five medium-sized to large, healthy, mongrel dogs, and were kept entirely free from the complicating factor of anesthesia. All procedures were comfortably and satisfactorily carried out without significant disturbance in the blood concentration by either emotional or muscular excitement. This required only a reasonable amount of attention to training well-nourished and well-disposed dogs.

In the experience of this laboratory, the simplest way to obtain a smooth base-line of blood concentration in dogs, is to keep the animals lying on one side, upon a comfortably padded table for at least one hour before the taking of blood samples. The only fixation is that of the upper hind leg, which is fastened in an extended position allowing easy access to the ankle vein when desired. The animal's front end must be kept uninterruptedly quiet by a persuasive attendant. Raising the head for an instant will definitely increase the blood concentration slightly, and this is the greatest muscular activity which can be tolerated. Better still is the absence of all visible movement, a goal nearly achieved in every experiment here reported.

The blood concentration was followed in all cases from blood freely flowing from the ear, the criterion being specific gravity determinations by the falling drop method. The standard fluid injection was 25 cc. per kilo of dextrose-free Ringer-Locke solution, all of the injections in any given dog being given by the same needle and occupying, with one exception (fig. 3) the same length of time. The fluid was introduced by gravity from an elevated reservoir through a heated coil, entering the vein at a temperature of about 40 degrees, or just above the dog's normal temperature. The skin over the ankle vein having been previously shaved and the leg fixed in extension, the only manipulations necessary at the time of injection were proximal compression of the vein, insertion of the needle, and release of flow. Sometimes a few cubic centimeters of blood were taken for sugar determination just before connecting the needle with the infusion system. After injection the leg was often entirely released for the remainder of the experiment.

No injection was begun before the animal had been quiet for considerably longer than one hour. The usual procedure was to follow the blood concentration for from twenty to forty minutes before the injection, and afterwards for about an hour.

When not fasted, the dogs were maintained upon our usual laboratory diet consisting of horse meat (Ken-L-Ration) and dog biscuits. Food was always withheld, even in the fed dogs, after 6 p.m. of the day preceding an experiment. Water was available at all times both during feeding and fasting, except when the animal was on the table.

The phlorhizin treatment in each case consisted of one injection each, on three consecutive days, of approximately 200 mgm. per kilo of Merck's phlorhizin, dissolved in two per cent sodium bicarbonate solution, (1 gram

phlorhizin to every 15 cc.). The injections were always made in the afternoon and subsequent to the day's observations.

RESULTS. *Effects of fasting with and without phlorhizin.* The effects of fasting and phlorhizin, independently of any fluid injections, are illustrated in figure 1. Each one of the points represents the average of several very careful determinations of specific gravity, made after the animal had been resting for at least an hour, as described above.

The upper curve shows the effect upon the blood concentration of dog 2, in which the specific gravity increased during five days' fast from 1.0551 to 1.0594 (7.8 per cent). The lower curve shows a similar increase in dog 3, amounting to 9.2 per cent after three days' fasting which was increased to 10.9 per cent after five days.

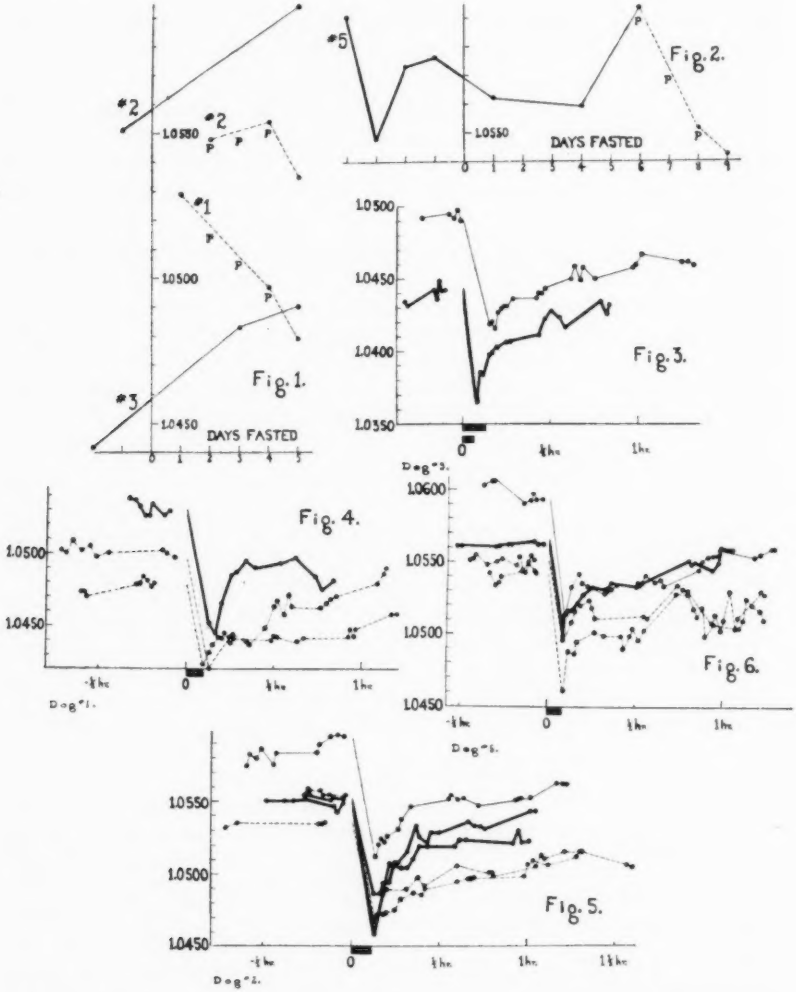
Dog 2 injected with phlorhizin (begun on the second day of fasting), showed a fall in specific gravity from 1.0548 on the second day to 1.0535 on the fifth day (2.4 per cent decrease), subsequent to a slight rise on the fourth day. In dog 1, the specific gravity after one day's fast was 1.0529, but with phlorhizin treatment fell to 1.0497 on the fourth day, and 1.0479 on the fifth day (decreases of 6.4 per cent and 10.4 per cent respectively).

Fasting anhydremia and phlorhizin hydremia having thus been demonstrated in separate experiments, a fresh dog was taken to see whether or not the latter condition could be superimposed upon a well developed fasting anhydremia. The results are illustrated in figure 2. The two determinations made just preceding the fast taken together with that on the first day of fasting, indicate that the dog's normal specific gravity was approximately 1.0570. Using this figure, it will be seen that, although no concentration had occurred by the fourth day, after the sixth day of fasting, the usual anhydremia was much in evidence (4.0 per cent increase). Phlorhizin treatment produced, as expected, a sudden shift to the hydremic condition. Two days' injection reduced the specific gravity to 1.0551, while on the next day it fell to 1.0542 (respective decreases of 3.4 per cent and 5.2 per cent below normal).

In figure 2 are also shown certain changes in specific gravity incident to external conditions. The first determination shows a high specific gravity, 1.0590, on the day after admission from a distant locality; in connection with the whole picture, this affords a good illustration of emotional anhydremia due to a strange environment. The next determination, 1.0548, made after a day's run in the yard, apparently owing in some way to fatigue, is decidedly below normal.

The experiments up to this point show that a fast of five or six days concentrates the blood of a normal dog, while if a similar or longer fast be accompanied by three days of phlorhizin injections, the blood exhibits, on the contrary, a tendency to hydremia. Although the serum of each phlorhizinized dog was twice examined for cloudiness, no such gross evidence of fat transport was found. Since the most extreme degrees of

lipemia recorded in the phlorhizin literature would not account for the observed specific gravity decrease one must regard the condition as "phlorhizin hydremia."



Effects of fluid injections in normal unanesthetized dogs. The intravenous injection of Ringer-Locke solution in the manner described above, produced a quite constant picture in normal dogs without anesthesia. This is illustrated by the heavy lines in figures 3, 4, 5 and 6 and by the table.

The maximum observed decrease in five different experiments ranged from 11.8 to 17.2 per cent and never persisted more than two or three minutes after cessation of the injection. Next occurred a sharp rise toward normal, becoming gradually less steep. At the end of fifteen minutes, the decrease ranged from 3.4 to 7.2 per cent (average 5.9 per cent). In the three experiments which were continued for a full hour, the decrease at the end of this period ranged from 0.5 to 4.7 per cent (average 2.3 per cent). (The trend of the discontinued curves supports this figure as typical for normal dogs under the conditions described.)

Effects of fluid injections in fasted dogs. The injections in fasted dogs are illustrated by the light unbroken lines in figures 3, 5 and 6. It will be seen that all of these curves are characterized by a delayed return to normal, illustrating a more prolonged retention of fluid in the circulation.

Fig. 1. Effects of fasting with and without phlorhizin upon the blood concentration of dogs. Abscissae: days before and after the beginning of fast; ordinates: blood specific gravity (average of several determinations); large numerals identify the animals. Dog 1, male hound, 19 kilos; dog 2, male hound 21½ kilos; dog 3, female collie, 7½ kilos. *P*, injection of phlorhizin.

Fig. 2. Effects of fasting and fasting plus phlorhizin, in dog 5, mongrel male, bull terrier, 14 kilos. Abscissae: days before and after beginning of fast; ordinates: blood specific gravity (average of several determinations). (First day's level attributed to strange environment, second day's to reaction after hard exercise.) *P*, injection of phlorhizin.

Fig. 3. Dog 3. Responses to intravenous injection of 25 cc. per kilo, dextrose-free Locke solution. Abscissae: time, related to beginning of injection; ordinates: blood specific gravity; heavy line: after normal feeding (duration of injection: lower block beginning with zero hour); light line: fifth day of fasting (duration of injection: upper block beginning with zero hour).

Fig. 4. Dog 1. Responses to intravenous injection of 25 cc. per kilo, dextrose-free Locke solution. Abscissae: time related to beginning of injection; ordinates: blood specific gravity; heavy line: after normal feeding; upper broken line: after two phlorhizin injections fourth day of fasting; lower broken line: after three phlorhizin injections fifth day of fasting. (Duration of all injections: block beginning with zero hour.)

Fig. 5. Responses to intravenous injection of 25 cc. per kilo, dextrose-free Locke solution. Abscissae: time related to beginning of injection; ordinates: blood specific gravity; both heavy lines: after normal feeding; (upper heavy line: 25 days later than lower line, and after recovery from intervening phlorhizin;) upper broken line: after two phlorhizin injections, fourth day of fasting; lower broken line: after three phlorhizin injections, fifth day of fasting; light continuous line: fourth day of fasting, six days after second normal experiment. (Duration of all injections indicated by block beginning at zero hour.)

Fig. 6. Dog 5. Responses to intravenous injection of 25 cc. per kilo; dextrose-free Locke solution. Abscissae: time related to beginning of injection; ordinates: blood specific gravity; heavy line: after normal feeding; light continuous line: sixth day of fasting; upper broken line: after two phlorhizin injections, eighth day of same fasting period; lower broken line: after three phlorhizin injections, ninth day of fasting. (Duration of all injections indicated by block beginning at zero hour.)

The maximal observed decrease was, of course, about the same as in normal dogs (average 14.1 per cent), while at the end of fifteen minutes, the retention of fluid is shown by decreases of 8.1 to 11.0 per cent (average 10 per cent). In no single case had the blood returned as near the normal as in any of the fed dogs. At the end of one hour, the decrease ranged from 5.3 to 6.2 per cent (average 5.9 per cent).

TABLE 1

Intravenous Ringer-Locke injections in resting unanesthetized dogs

DOG NUMBER	INITIAL BLOOD SPECIFIC GRAVITY	MAXIMUM OBSERVED DECREASE	DECREASE 15 MINUTES AFTER INJECTION	DECREASE 1 HOUR AFTER INJECTION	SERUM SUGAR
Normal					
		per cent	*per cent*	*per cent*	*per cent*
1	1.0529	15.9	7.0		
2a	1.0548	16.2	6.2	4.7	
2b	1.0551	11.8	3.4	1.6	
3	1.0442	17.2	7.2		
5	1.0562	11.4	5.5	0.5	
Average		14.5	**5.9**	**2.3**	
Fasting					
2	1.0594	13.8	8.1	6.1	
3	1.0490	15.1	11.0	5.3	
5	1.0593	13.5	10.8	6.2	
Average		14.1	**10.0**	**5.9**	
Fasting and phlorhizin					
1a	1.0497	15.5	11.9	2.8	0.05
1b	1.0479	11.7	8.1	5.2	0.12
2a	1.0554	15.5	10.7	7.8	0.11
2b	1.0535	12.7	9.2	5.4	0.06
5a	1.0551	9.9	7.2	7.1	0.08
5b	1.0542	14.9	7.9	6.8	0.10
Average		13.4	**9.2**	**5.9**	

Effects of intravenous injections in fasted dogs after phlorhizin treatment. Six experiments were made upon fasted dogs after subjection to a three days' course of phlorhizin injections. In each of three dogs, two experiments are illustrated by the light broken lines in figures 4, 5 and 6 respectively. Starting from levels indicating hydremia in all except one experiment one sees a maximal density decrease ranging from 9.9 to 15.5 (average 13.4 per cent). This figure agrees closely with those for both

types of non-phlorhizinized dogs. The return to the pre-injection level was conspicuously slow in all six experiments, notwithstanding the fact that this level itself was a low one. At the end of fifteen minutes the decrease ranged from 7.2 to 11.9 per cent (average 9.2 per cent), while after sixty minutes, it ranged from 2.8 per cent to 7.8 per cent (average 5.9 per cent). Thus the results in the fasting but non-phlorhizinized dogs were practically duplicated.

In all six experiments upon phlorhizinized dogs, serum sugar determinations were made from blood samples taken just before the fluid injections. From the results included in the table, it will be seen that the stage of hypoglycemia characteristic of phlorhizin happened to be present in three out of the six instances (0.5 to 0.8 per cent serum sugar). There was nothing in the response to fluid injection which tended to differentiate these three experiments from the other three on the same dogs, in which the serum sugar was normal. The conclusion is therefore drawn that fluid retention in the circulation after phlorhizin treatment is not determined by the blood sugar level.

Besides the demonstration of hypoglycemia in all three cases, evidence of typical phlorhizin poisoning was found in the fatty livers of dogs 1 and 2. The first dog was sacrificed and the liver showed extreme fatty degeneration on inspection (confirmed histologically). In dog 2, the liver was directly inspected through a small opening made under local anesthesia, a day or two after the last phlorhizin injection, and found to present a similar appearance to that of the first dog. (Dog 5 was allowed to recover without liver examination.)

Discussion. The abnormally long retention of fluid observed in the dogs merely fasted, might perhaps have been attributed to the relatively dry condition of the blood. But in bloods initially diluted by phlorhizin we found just the same degree of retention. One must therefore seek an explanation elsewhere than in the condition of the blood.

This brings us to a consideration of the viewpoint from which the investigation started, namely, the possible influence of a fatty liver upon the retention of fluid in the blood. Inasmuch as we found a like result in fasted dogs regardless of the presence or absence of phlorhizin poisoning, it is necessary to consider only those features common to the two conditions, that is, those due to fasting. Lusk (1923) states that fasted dogs may accumulate fat in the liver amounting to 10 per cent of the weight of this organ, while the fat in the phlorhizin liver sometimes amounts to 30 per cent.

Just as important perhaps as the liver condition is presumably the condition of the tissues in general. After five or more days of fasting, the shifting of fat from depots to active cells would have an effect upon their behavior toward an offering of extra fluid. Evidence on this point is

afforded by the striking results of Kleitman who finds that dogs voluntarily drink far less water when fasted than when fed.

According to investigations of Krause (1922) (working with Carlson) the tissues of fasted pigeons tend to acquire water. A striking exception was the spleen which loses over 15 per cent of its water,—very likely a reflection of the condition of the contained blood. Others also find a tendency to edema in some organs of fasted animals. Biddle and Howe (1912) found the muscles of fasted dogs richer in water, while Szörényi (1927) has recently made the observation that fasted white mice exhibit an increase in the water percentage of the entire animal. Edema suggests lessened avidity for injected fluid. The bearing of these findings upon our work is perhaps too obscure for further immediate discussion.

It is hoped that the present contribution will help to show the need for a thorough investigation of those factors which render tissues more resistant to injected fluid. Under the conditions of shock, means should be devised to combat the tendency of the tissues to deprive the circulation of its vital need.

SUMMARY AND CONCLUSIONS

1. A fast of five or six days induced anhydremia in normal dogs.

2. A similar or longer fast accompanied by a course of phlorhizin injections produced on the contrary a tendency toward hydremia. There was no gross evidence of lipemia.

3. Fasted dogs retained in the circulation more fluid one quarter hour and one hour after intravenous injections of dextrose-free Locke solution than normal dogs. The same degree of retention was noted under phlorhizin, both in and out of the phase of hypoglycemia.

4. The tissues of fasted animals (with or without phlorhizin) are less avid of intravenously injected fluid than during normal feeding.

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FACTORS WHICH DETERMINE RENAL WEIGHT[1]

I. Methods

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The growth of the kidney is in the main dependent on the factors which determine the growth of the body as a whole. This fact is demonstrated by the high correlation which is found between the weight of the kidneys and such indications of general body growth as are given by measurements of body weight, body length and body surface (Jackson, 1913; Hatai, 1913). But the rate of growth of the kidney can be accelerated or inhibited by the introduction of factors which have little or no effect on growth in general. During the last four years data which partially define the effect of these specific determinants of renal growth and maintenance have been collected and are given in this series of papers in the hope that they may ultimately contribute to a more complete understanding of the mechanism of the changes in renal weight which occur during extra-uterine life.

The control and experimental animals were matched on the basis of age, sex and body surface. The food intake was measured. The temperature, lighting, housing, and handling were constant. If any organ was removed from the experimental rats the same organ was exposed in the control animals. All such variables can be eliminated or measured, but there remain many others which are not susceptible of control, and no exact agreement in the results of duplicate experiments is attainable. We therefore dealt with such a number of animals that a reliable statistical measure of the probable range of this intrinsic variability might be computed. This paper gives the exact details of the methods which were used.

Experimental subject. The rats which formed the nucleus of the colony used for these experiments were obtained from Prof. J. R. Slonaker of the Physiology Department of Stanford University. This strain has the same origin as the present albino stock of the Wistar Institute in Philadel-

[1] This work was aided by the Wellington Gregg Fund for the Investigation of Bright's disease.

[2] The data in this series of papers were taken from the dissertation presented by this author in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Stanford University, 1926.

phia. Both strains originated from a colony which was maintained in the Neurological Laboratory at the University of Chicago from 1900 to 1907. Dr. Slonaker transferred the nucleus of his colony to California in 1903 and in 1907 some of these animals were brought to Philadelphia and have served as a basis for the Wistar Colony. The Slonaker stock have been inbred ever since 1903 and are a very uniform healthy stock of which the average age-weight curves not only compare very favorably with the original curves of Donaldson (1924a) but in most instances are somewhat superior.

To maintain the colony, it was made a part of the routine of numbering the rats for the different experiments to set aside from time to time several cages of unselected females to be used for breeding stock. These rats were not mated until they had attained 135 days of age. It was our usual custom to discard a female after the fourth litter. The young were removed from their mothers when 25 days of age. Every rat unless obviously malformed, undernourished, or diseased was marked serially and assigned to an experiment, whether immediate or future, or to breeding and the groups arranged at this time. A record of the mother's number, litter number, exact birth date, sex, cage and experiment number was kept for each rat. As will be noted above no selection as to size, vigor or other characteristic was resorted to in the grouping of rats for any purpose. If possible it is very desirable to have a group of rats selected for a given age at one time compared with groups of the same age taken from the colony at any other time. On this account no selection of any kind other than age was ever resorted to. At all times an attempt was made to keep the "average rat" the same.

EXPERIMENTAL ENVIRONMENT. *Room.* All experiments were carried on in a room ventilated by forced air from outdoors and passed over steam pipes which maintained a temperature of approximately 23°C. by means of an automatic regulator. During the warm weather of late September it was not possible to maintain the temperature at 23°C. but it never exceeded 29°C. even then; and in all, not less than 90 per cent of the time the temperature was constant at 23°C. within 1.5° in either direction. No attempt was made to regulate the moisture content of the atmosphere but daily observations of the relative humidity over a period of five months were remarkably constant. The light was about the same intensity thoughout the room and all rays had to pass through window glass. Rats placed in experiments at 30 days of age were in this special room from birth and all others for 15 days before being placed in an experiment. All rats which were not to be used in an experiment when 30 days old were transferred to a well ventilated stock room where the temperature ranged from 19° to 26°C. A minimal amount of light enters this stock room.

Cages. The double cage described by Slonaker (1918) was used for both the stock colony and to house our experimental animals. These cages are constructed of a galvanized iron frame and netting and rest on gal-

vanized sheet iron bottoms. The latter were painted with asphaltum varnish to prevent rusting but did not come in contact with the rats as they were covered with shavings. An inverted bottle furnished each cage with fresh tap water.

Caging. In the stock colony from six to eight rats were kept in each side of a double cage. The experimental animals were divided into groups of four to six. All cages were provided with bedding to a depth of several centimeters in the form of rather coarse, clean pine shavings. In addition in each stock cage a couple of handfulls of sawdust, which had been previously moistened with 5 per cent sulfuric acid and dried, were added to the bedding when it was cleaned. Every cage was cleaned at least once each week and the cages containing experimental animals usually oftener.

Feeders. Two types of feeders were used. Both kinds fit on the door of the Slonaker cage. Figure 1 is a diagram of the feeder which was used for the stock cages. These were not removed from the cage door for filling but

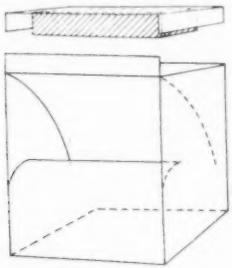

Fig. 1

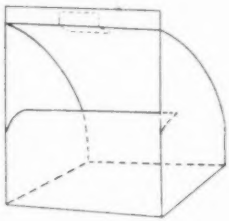

Fig. 2

were filled from the back by removal of the cover. The stock food was dry and easily fell into the feeder proper as it was emptied. All feeders were emptied and cleaned with a brush and boiling water from time to time.

For the experimental cages a somewhat different type of feeder was preferred. It is sketched in figure 2. The food mixtures were packed in rather solidly by pressing with one's fingers or a spatula so that the occasional shavings which were kicked in might be shaken out without loss of food. In the case of a dry powdery food only a shallow layer (3 cm. deep) could be placed in a feeder if none was to be thrown out.

Stock diet. Greenman and Duhring (1923) and more recently Osborne and Mendel (1926) have shown that a marked change may take place in a strain of albino rats through the influence of simple dietary changes. Variation of this sort was anticipated and was the very kind which we wished to avoid and so our diet has been kept constant for stock rats of all ages the year around. Steenbock has described (1923) a very satisfactory

stock diet for rats which includes the use of fresh milk, and suggested certain modifications in case milk was not fed in addition. Following his suggestions in part a modification of this diet was used. The ingredients of the diet and its composition are given in table 1. It was found unnecessary to prepare fresh stock food oftener than once a week but food over a week old was never used. This stock diet has an energy value of about 3.97 calories per gram. It has proven adequate for normal growth, maintenance and reproduction in a colony of some two thousand rats over a period of four years.

TABLE 1

Composition of stock diet

INGREDIENT	TOTAL	PROTEIN*	FAT	CARBOHYDRATE	WATER	ROUGHAGE	SALTS	CALORIES
Feed corn meal	68.0	6.3	2.7	47.5	8.5	2.0	0.9	244.9
Linseed oil cake meal	10.0	3.0	0.9	3.5	1.1	0.9	0.6	35.0
Dried ground alfalfa	2.0	0.3	0.2	0.5	0.2	0.6	0.2	5.1
Powdered casein	10.0	8.3	0.1	0.5	0.9	0.0	0.2	37.1
Lard	5.0	0.0	5.0	0.0	0.0	0.0	0.0	46.5
Cod-liver oil	3.0	0.0	3.0	0.0	0.0	0.0	0.0	27.9
Bone ash	1.5	0.0	0.0	0.0	0.0	0.0	1.5	0.0
Sodium chloride	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0
Total	100.0	17.9	11.9	52.0	10.7	3.5	3.9	396.5
Calories		72	111	213	0	0	0	396.5

ELEMENT	PER CENT OF DIET	PER CENT OF CALORIES
Protein	17.9	18.4
Fat	11.9	28.0
Carbohydrate	52.0	53.6
Water	10.7	0 0
Roughage	3.5	0 0
Salts	3.9	0 0

* Total N × 6.25.

EXPERIMENTAL PROCEDURE. *Standard experimental diets.* During all experimental periods modifications of the casein-starch-lard diets of Osborne and Mendel were used. All cornstarch was cooked and the jelly dried in a current of hot air. The dried product ground to a fine powder constituted the "cornstarch" of our experimental foods. Finely powdered commercial casein formed the source of protein. The lard, cod-liver oil and agar were the products ordinarily obtainable on the market. The dried powdered yeast was obtained from a constant source (the Harris Laboratories, Tuckahoe, N. Y.) and had been tested for its vitamine B activity.

Since the yeast was fed not separately but as one of the ingredients mixed in the food it was found necessary to use a rather high percentage in the male diet. In the female diet the wheat germ appeared to compensate for the smaller yeast content. The salt mixture was that used by Osborne and Mendel (1918) based on the composition of the salts in milk. It was prepared (without lactose) from Baker's C.P. carbonates and acids and dried in a current of air at 110°C.

All of the dry ingredients of the experimental diets, with the exception of the wheat germ in the female diet, were reduced to such a degree of fineness

TABLE 2

Composition of experimental male diet

INGREDIENT	TOTAL	PROTEIN	FAT	CARBOHYDRATE	WATER	ROUGHAGE	SALTS	CALORIES
Cornstarch	45.0	0.0	0.0	41.4	3.6	0.0	0.0	169.7
Casein	16.0	13.3	0.2	0.7	1.4	0.0	0.4	59.3
Lard	15.0	0.0	15.0	0.0	0.0	0.0	0.0	139.5
Cod-liver oil	9.0	0.0	9.0	0.0	0.0	0.0	0.0	83.7
Salt mixture (Osborne and Mendel)	4.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0
Dried yeast	9.0	4.7	0.0	0.0	0.4	2.9	1.0	19.3
Agar-agar	2.0	0.0	0.0	0.0	0.2	1.8	0.0	0.0
Total	100.0	18.0	24.2	42.1	5.6	4.7	5.4	471.5
Calories		74	225	173	0	0	0	471.5

ELEMENT	PER CENT OF DIET	PER CENT OF CALORIES
Protein	18.0	15.7
Fat	24.2	47.7
Carbohydrate	42.1	36.6
Water	5.6	0.0
Roughage	4.7	0.0
Salts	5.4	0.0

that they would pass through a number 30 (U. S. Standard) mesh sieve. The dry materials, followed by the oil and lard, were finely mixed by means of a machine-driven dough mixer. All experimental diets were prepared in fresh batches at least once a week. The composition of the standard experimental male food is outlined in table 2.

As we desired to perform certain experiments concerning the effect of pregnancy it was necessary to include an adequate source of vitamine E (Evans and Bishop, 1922) in our experimental female diet. At the suggestion of Dr. H. M. Evans of the University of California this was in-

cluded in the form of wheat germ (specially prepared) which formed 10 per cent of the female diet. We have since found that the male diet contains the fertility factor in adequate amounts. The ingredients and composition of the female diet are given in table 3.

The majority of the experiments were carried out with the standard male and female diets which have just been described. Except for the very young rats these animals had been on the stock diet for some time. In

TABLE 3

Composition of experimental female diet

INGREDIENT	TOTAL	PROTEIN	FAT	CARBOHYDRATE	WATER	ROUGHAGE	SALTS	CALORIES
Cornstarch	41.0	0.0	0.0	37.7	3.3	0.0	0.0	154.6
Casein	15.0	12.5	0.2	0.6	1.4	0.0	0.4	55.6
Lard	15.0	0.0	15.0	0.0	0.0	0.0	0.0	139.5
Cod-liver oil	9.0	0.0	9.0	0.0	0.0	0.0	0.0	83.7
Salt mixture (Osborne and Mendel)	4.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0
Dried yeast	4.0	2.1	0.0	0.0	0.2	1.2	0.4	8.6
Wheat germ	10.0	2.6	0.9	4.4	1.3	0.4	0.4	37.1
Agar-agar	2.0	0.0	0.0	0.0	0.2	1.8	0.0	0.0
Total	100.0	17.2	25.1	42.7	6.3	3.4	5.2	479.1
Calories		71	233	175	0	0	0	479.1

ELEMENT	PER CENT OF DIET	PER CENT OF CALORIES
Protein	17.2	14.8
Fat	25.1	48.6
Carbohydrate	42.7	36.6
Water	6.3	0.0
Roughage	3.4	0.0
Salts	5.2	0.0

figures 3 and 4 the composition and caloric values of these diets have been compared.

Routine. In the primary grouping of experimental rats selection was made on the basis of age and sex. Each experiment was carried out with 24 to 50 rats. As mentioned before, the male rats were separated from the females at the time when they were marked and set aside in groups to await the dates for which they were planned for experiments. When this time came and a group was finally arranged for an experiment the litters (males and females) were listed in order of birth date and introduced into the experiment in the correct order.

No rat was weighed before this time. After being placed in an experiment each rat was weighed daily at about the same time each day to within 1 gram. As has been pointed out, change in body weight was not a point of experiment and since individual figures are not necessary the average weight of an experimental group could have been determined en masse. Certain other factors, however, such as the somewhat different

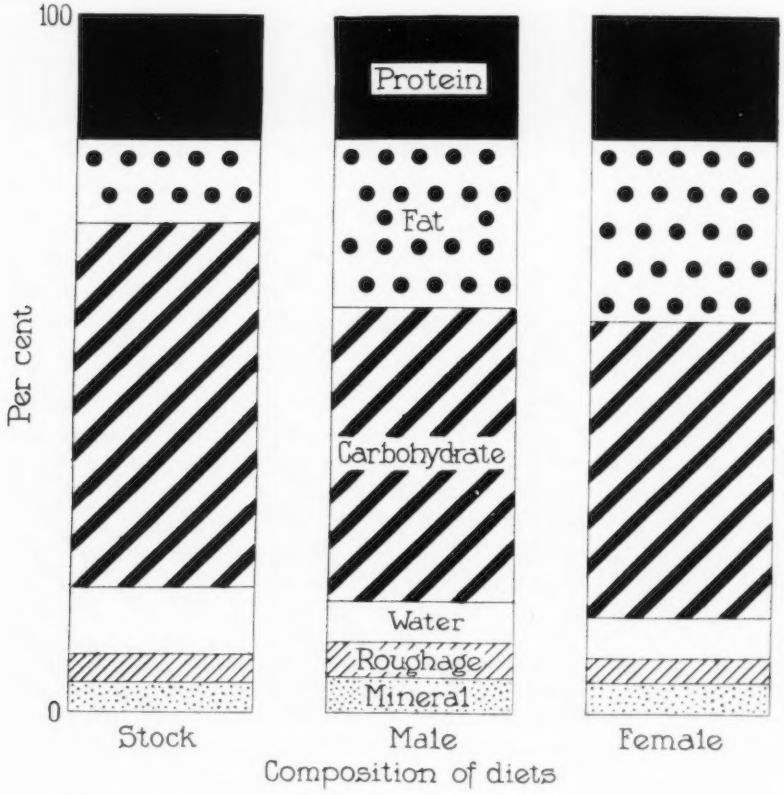

Fig. 3

ages of the members of a group and the value of body weight estimations as an index of the condition of a rat made individual and frequent weighings a necessity.

When an experiment was started the feeder for each cage was filled with the experimental diet and its initial weight taken and the daily food intake determined from then on. When food intake was considered, the objec-

tions to group determinations of body weight, which is also an accessory factor, did not arise and the method of group feeding and determination of food intake which will be described in the next paragraph was resorted to.

The food intake was determined on cage units of from four to six rats for two day intervals by means of the feeder previously described. It was assumed that each rat in a cage ate approximately the same amount of

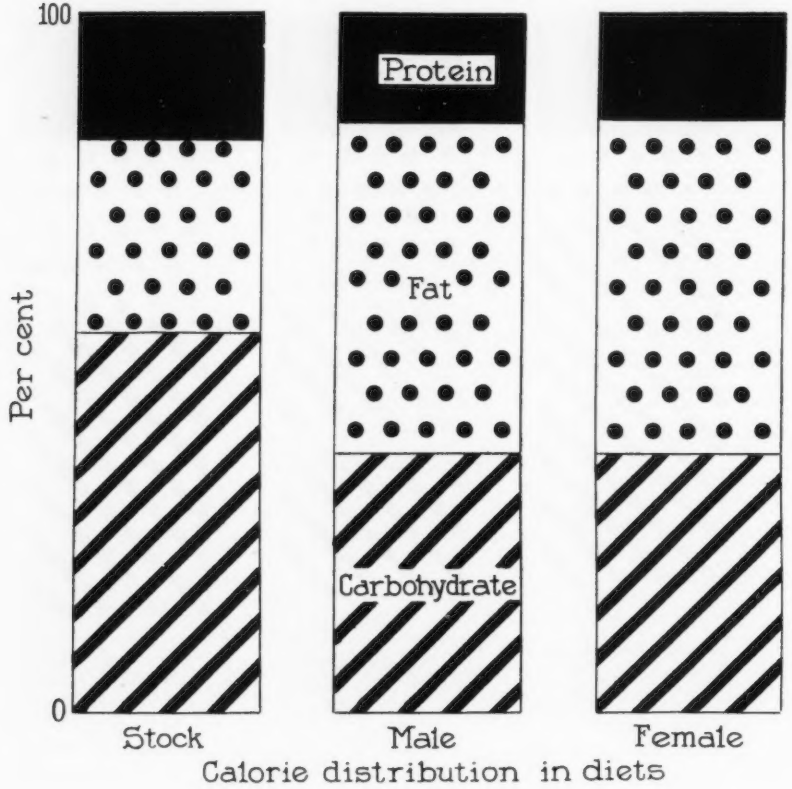

Fig. 4

food over this two day period and therefore it was essential that all the animals in a given cage were near the same day of an experiment. Since the food intake was not determined individually close check had to be kept on the body weight of each rat as an index of a possible insufficiency in its food intake. It was necessary to remove from a group any rat which showed weight changes obviously different from the rest. The feeders were weighed to within one gram involving an error of 1 to 2 per cent.

In many instances all of the rats to go in a given cage were not exactly the same age and so were not introduced into the cage at the same time, but serially as each one reached the proper date for its entrance into the experiment. It was essential therefore, whatever other days a feeder was to be weighed, that it be weighed each time a new rat was added to a cage or that one was removed from a cage during the closing days of an experiment.

Daily weighing and food intake determinations were always continued until the time planned for the termination of an experiment. At this time the rats were killed, serially just as they were introduced into the experiment, and the kidneys and certain other organs weighed and preserved. This procedure will be described later. It should be emphasized that the entrance of every rat into an experiment and killings were all carried out at exact days of age. The necessity for such accuracy must be evident when one considers the relation of a single day to the life span of a rat.

At the end of each experiment the daily body weights and the daily food intake for the separate groups were averaged and charted for comparison with other experiments.

Killing. Each experiment was brought to an end by killing the rats in it on exact days of age. The "killings" were done as nearly as possible at the same time every day. The procedure was as follows: the rat was first weighed to within 1.0 gram, this weight being recorded as the "gross" body weight, and then put in the etherization bottle. On its removal from the bottle the body length of the animal was obtained by laying the rat on its back on a millimeter ruler and firmly stretching it out to its full length. The distance from the tip of the nose to the center of the anus was found by means of pointers and its value in millimeters recorded as body length. The length determined in this manner is not regulated by the strength of the pull used in stretching the rat out as it is apt to be when the lifeless eviscerated body is extended on a ruler. This measurement was always made with the aid of an assistant. Ether administration was then resumed. The abdomen and thorax were moistened with 95 per cent alcohol which served to keep the hair from flying about and adhering to the viscera and instruments. A longitudinal incision was then made through the skin and muscle from the pubis to the ensiform cartilage and a short cut at right angles to this one made on each side into the abdominal wall in order to facilitate the handling of the organs. The intestine was cut as far as could be reached toward the rectum and pulled away so as to expose the posterior wall of the abdominal cavity. To obtain the rat's blood the animal was tilted with its head up and, with a test tube containing a small amount of powdered potassium oxalate placed against the vertebral column, the vein and artery cut just above the bifurcation of the abdominal aorta. Two to 5 cc. of blood were thus obtained easily in a few seconds. Urea and other substances were determined on these blood specimens. A wad of fine cotton was then pushed into the bleeding area and left there until the kidneys were removed.

To do this the intestine and spleen were pushed aside and the left kidney grasped with the blunt ring forceps and cut away widely so that there was no chance of injury. A wad of cotton was then pushed into this area and the right kidney immediately removed in the same manner. The assistant received the two kidneys on a piece of filter paper and prepared them for weighing, first the left one and then the right one. Each kidney was decapsulated with a finely pointed bent dissecting needle. The capsule and vessels adhere to the filter paper and, if the kidney be gently raised, pull away from the hilus so that they can be neatly clipped off at this point with the straight iris scissors. Two parallel longitudinal cuts were made to either side of the mid-line of the kidney dividing it into three slices. The three slices were blotted between layers of filter paper to get rid of any blood and urine which could be pressed out. Whatman's no. 42 filter paper was found best for this purpose, especially as the tissue does not adhere to it. The slices after being blotted were quickly weighed either separately or two or three at a time depending on their weight. This method of blotting the pieces of kidney before weighing them was decided upon as the fairest and most accurate means of obtaining weights which represented renal tissue alone and to eliminate the inclusion of the weight of blood and urine which might be present in the kidney at the time of its removal and which could never be regulated so as to be constant.

The weighing was done on a Hartmann-Braun torsion balance, a dial instrument with which accurate weights could be obtained in milligrams within a few seconds. Thus although the tissue was exposed while being weighed, drying had no observable effect during the extremely short time required. Two torsion balances were used depending on the size of the kidneys being handled, one of 500 mgm. capacity with a dial in 500 divisions, and the other of a capacity of 1000 mgm. with the scale divided into 500 divisions of 2 mgm. each. The accuracy of the weighing was very close to 0.5 per cent in every case. The weight of each kidney from every animal was recorded in milligrams. The slices comprising each single kidney were preserved in 10 per cent neutral formalin in small individual vials.

While the assistant prepared and weighed the kidneys the operator removed the cotton sponges which had been used to absorb the excess blood and then proceeded to remove the entire intestinal tract up to and including the lowest part of the esophagus. A snip of the mesentery of the cecum was necessary but the rest of the intestine up to the duodenum was easily freed of its mesentery by drawing it against the scissors. When the stomach was reached it was necessary to cut away the pancreas and lesser omentum. The esophagus was clipped off and the entire intestinal tract thus removed was weighed in the pan of a small triple beam balance to within half a gram. The result was recorded in whole grams. The intestine was discarded and

the liver then cut free of its ligaments and vessels and any adhering blood wiped away with smooth cotton. The liver was then weighed on a beam balance to within 0.02 gram and the weight recorded. Following this a cut was made through the costal cartilages on the right side from the abdominal cavity to the clavicle. The heart could then be grasped at the apex, torn out of the pericardial sac, and drawn outside. The vessels were easily clipped off just outside the auricles. The heart, which was usually still beating, was cut in two and the two halves pressed within a folded pad of soft cotton. The heart tissue was then weighed immediately by the assistant on the torsion balance and discarded. The weight was recorded in milligrams. The carcass of the rat was then thrown away.

The whole procedure of killing a rat and weighing these several organs, as has just been described, seldom required more than seven minutes and often as few as four. Two persons always worked together.

EXPERIMENTAL DATA. *Kidney weight.* The kidney weight which will be used is the average (in millligrams) of the two organs in each rat. Although the method by which this weight was determined has been described some comment on it is necessary. In determining the weight of the kidney tissue the capsule was of course removed as being distinct from the functional renal tissue. The weight of a decapsulated kidney includes other extraneous material which is not a part of, and bears no constant relation to, the amount of active tissue,—that is, the contained urine and blood. Although these cannot be entirely removed from the kidney tissue the quantity can be reduced so that it no longer causes an important error. This reduction was attained by slicing the kidneys longitudinally as has been described and firmly blotting them on special filter paper. As it was found impossible to squeeze out further fluid by cutting these blotted slices into still thinner ones and repeating the blotting we think that one may safely assume that practically all of the excess blood and urine has been removed from the kidney tissue when this procedure is carried out immediately on the removal of the kidney and before the blood has become clotted. The very small amount of fluid which remains is probably a factor which is determined by the amount of tissue and has a constant relation to it. This removal of excess blood and urine has proven very important especially in the case of the large watery kidneys obtained by feeding a high urea or protein diet. Here, for instance, a very large decrease in the apparent weight of a kidney is brought about by blotting the slices of tissue.

Body weight. Jackson and Lowrey (1912) expressed the true body weight as the "net weight" (that is, body weight minus the weight of the intestinal contents). This figure however is obtained only with difficulty and the necessary procedures are out of the question when large numbers of observations are to be made. We have substituted a calculated weight which does not differ significantly from this true net weight.

It is determined as follows: the weight of the intestinal tract and its contents is subtracted from the gross body weight; the intestinal tract bears a percentage relationship to this difference and this percentage was determined from Donaldson's table (1924b) for a rat of the given age and sex; this percentage is then taken of the gross body weight minus the weight of the gastro-intestinal tract and its contents and added to it. The result in grams is the figure we have used for body weight and called the "corrected body weight."

Body length. The distance in millimeters from the tip of the nose to the center of the anus, found by the methods described, is the body length.

Body surface. Meeh's formula for body surface wherein this measurement is determined by multiplying the two-thirds power of the body weight by a constant, K, has been found to hold for the albino rat when K is 11.36 (Carman and Mitchell, 1926). Our figures for body surface have been obtained on this basis using for the body weight figure the corrected body weight described above.

SUMMARY

A description is given of the means used to approximate constant conditions in a colony of albino rats and of the methods employed in making anatomical measurements.

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FACTORS WHICH DETERMINE RENAL WEIGHT[1]

II. Age

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The size of a rat is in general determined by its age. Since the weight of the kidneys tends to be a function of the size of the animal young rats naturally have kidneys which weigh less than those of older rats. This is to be expected and does not indicate that age, apart from its effect on body size, has any influence on renal weight. It is essential that the weight of the kidneys at different ages be considered in relation to the size of the rat. A relationship varying with age is suggested by Donaldson's formulaeic tables (1924) of kidney weight on body weight and length. Experiments are described here which were designed to examine this point.

The data were all obtained from male rats by methods described in the preceding paper (MacKay and MacKay, 1927). In a given group every animal was of exactly the same age. The rats received the special diet for at least 9 days and in most of the groups for 54 days preceding death. A comparison was made of the kidney weight of every rat at each age in relation to the amount of kidney tissue per unit of the body length, weight and surface. The results are given in table 1. In this table the mean ratios of the kidney weight to the various standards at each age have been summarized with their statistical significance. These means have been plotted against age in figure 1. Each mean has been fitted with wings, the spread of which on each side of the point covers a range of four times the probable error. This range includes almost all of the results which might have been obtained by numerous repetitions of the experiment.

It is evident from figure 1 that kidney weight in relation to body weight decreases with increasing age and reaches a minimum value at about 200 days of age. The weight of the kidney in relation to body length on the other hand increases with age, attaining a maximum value at about the same age. The differences found in the effect of age on the kidney weight depending on whether body weight or length is used as a reference standard are due to the fact that body weight has a higher and body length

[1] This work was aided by the Wellington Gregg Fund for the Investigation of Bright's disease.

TABLE 1

AGE	NUMBER OF RATS IN GROUP	AGE GROUP MEANS				KIDNEY WEIGHT PER								
						100 grams body weight			100 mm. body length			100 sq.cm. body surface		
		Kidney weight	Body weight	Body length	Body surface	Mean M	Standard deviation σ	Coefficient of variation v	Mean M	Standard deviation σ	Coefficient of variation v	Mean M	Standard deviation σ	Coefficient of variation v
days		*mgm.*	*grams*	*mm.*	*sq.cm.*	*mgm.*	*mgm.*		*mgm.*	*mgm.*		*mgm.*	*mgm.*	
35	18	313	60	143	175	524±10.8	67.5±7.6	12.9±1.5	219±4.6	28.9±3.3	13.2±1.5	180±3.3	20.5±2.3	11.4±1.3
40	19	433	86	157	219	506±8.3	53.8±5.9	10.6±1.2	276±3.8	24.7±2.7	9.0±1.0	197±2.6	16.6±1.8	8.4±0.9
45	18	460	95	163	235	486±6.4	39.4±4.4	8.1±0.9	283±5.7	36.1±4.1	12.8±1.4	195±2.6	16.2±1.8	8.3±0.9
50	17	493	111	169	261	444±6.0	24.5±2.8	5.7±0.7	291±5.3	32.6±3.8	11.2±1.3	187±2.2	13.1±1.5	7.0±0.8
60	17	595	137	183	302	437±7.4	44.4±5.1	10.2±1.2	325±5.6	34.0±3.9	10.5±1.2	197±2.8	17.1±2.0	8.7±1.0
70	25	644	170	192	348	380±3.0	22.6±2.2	5.9±0.6	335±2.7	20.1±1.9	6.0±0.6	185±1.3	10.0±1.0	5.4±0.5
80	17	807	225	209	420	358±3.3	20.4±2.4	5.7±0.7	386±6.1	37.2±4.3	9.6±1.1	192±1.8	11.1±1.3	5.8±0.7
90	18	790	228	209	438	362±4.0	24.8±2.8	6.9±0.8	378±3.2	19.8±2.2	5.2±0.6	191±2.2	13.8±1.5	7.2±0.8
95	12	757	225	210	420	337±3.4	17.4±2.4	5.2±0.7	360±4.2	21.5±3.0	6.0±0.8	180±1.7	8.7±1.2	4.8±0.7
100	12	849	253	214	454	336±4.6	23.8±3.3	7.1±1.0	397±4.4	22.6±3.1	5.7±0.8	187±2.3	11.9±1.6	6.4±0.9
105	12	811	250	211	451	325±4.1	21.2±2.9	6.5±0.9	384±4.0	20.7±2.8	5.4±0.7	180±1.9	9.6±1.3	5.3±0.7
110	12	843	250	213	450	339±3.7	19.2±2.6	5.2±7.2	396±6.6	34.0±4.7	8.6±1.2	187±1.7	8.6±1.2	4.6±0.6
120	28	873	268	218	471	328±3.1	24.3±2.2	7.4±0.7	400±4.6	36.0±3.2	9.0±0.8	185±1.7	13.1±1.2	7.1±0.6
130	25	850	265	214	469	321±3.4	25.1±2.4	7.8±0.8	394±5.3	39.2±3.7	9.9±1.0	181±1.8	13.2±1.3	7.3±0.7
140	12	914	329	225	530	279±1.9	9.8±1.3	3.5±0.5	406±6.3	32.2±4.4	7.9±1.1	169±1.0	5.3±0.7	3.1±0.4
150	13	1047	327	226	554	322±5.3	28.2±3.7	8.8±1.2	428±5.7	30.5±4.0	7.1±0.9	189±2.5	13.5±1.8	7.1±1.0
180	12	1019	346	232	559	295±4.3	22.2±3.1	7.5±1.0	439±5.8	29.7±4.1	6.8±0.9	182±2.3	11.7±1.6	6.4±0.9
185	12	860	314	226	523	276±6.2	31.8±4.4	11.5±1.6	379±6.9	35.5±4.9	9.5±1.3	168±4.3	22.3±3.1	13.3±1.8
190	12	888	326	229	538	275±4.9	25.3±3.5	9.2±1.3	388±4.0	20.4±2.8	5.3±0.7	166±2.0	10.5±1.4	6.3±0.9
195	12	852	306	224	516	280±4.1	20.8±2.9	7.4±1.0	380±4.5	22.8±3.1	6.0±0.8	167±2.5	12.7±1.7	7.6±1.0
200	11	856	298	222	492	288±2.8	13.8±2.0	4.8±0.8	412±2.4	11.8±1.7	2.9±0.4	174±1.2	5.7±0.8	3.3±0.5
210	11	898	306	229	515	294±3.2	15.7±2.3	5.4±0.8	392±8.7	42.7±6.1	10.9±1.6	174±2.1	10.1±1.5	5.8±0.8
220	24	986	361	231	576	274±2.2	16.0±1.6	5.8±0.6	428±4.3	31.3±3.1	7.3±0.7	172±1.4	9.9±1.0	5.8±0.6
230	12	935	355	227	569	275±8.5	43.9±6.0	16.0±2.2	412±4.9	24.9±3.4	6.1±0.8	165±2.8	14.2±2.8	8.6±1.2
310	27	976	381	234	596	257±2.8	21.8±2.0	8.5±0.8	416±4.0	30.8±2.8	7.4±0.7	164±1.4	10.8±1.0	6.6±0.6
365	13	988	390	236	605	257±5.6	29.7±3.9	11.5±1.5	421±5.2	27.8±3.7	6.6±0.9	164±2.3	12.2±1.6	7.4±1.0
370	12	994	384	227	599	261±4.7	24.3±3.3	9.3±1.3	418±4.5	23.1±3.2	5.5±0.8	166±2.1	10.6±1.5	6.4±0.7
375	12	944	321	225	532	297±5.0	25.7±3.5	8.7±1.2	421±5.7	29.3±4.0	7.0±1.0	179±1.9	9.8±1.4	5.5±0.8
380	12	1002	352	232	566	288±5.7	29.2±4.0	10.1±1.4	431±5.1	25.9±3.6	6.0±0.8	178±2.3	11.8±1.6	6.6±0.9
390	12	1006	396	238	610	257±5.0	28.8±4.0	10.2±1.4	422±0.7	37.6±5.2	8.9±1.2	165±2.3	12.0±1.6	7.3±1.0
400	22	1035	367	231	582	283±4.2	29.1±3.0	10.3±1.0	448±8.5	39.8±4.0	8.9±0.9	178±2.3	15.7±1.6	8.8±0.9
410	12	1001	391	238	608	258±4.4	22.4±3.1	8.7±1.2	420±4.6	23.5±3.2	5.6±0.8	165±1.7	8.6±1.2	5.2±0.7
580	23	1101	430	238	642	267±4.2	31.1±3.1	11.3±1.1	459±5.6	39.9±4.0	8.8±0.9	172±2.1	15.2±1.5	8.8±0.9
760	22	1025	341	233	553	305±5.5	38.5±3.9	12.6±1.3	440±4.3	30.0±3.1	6.8±0.7	186±2.5	17.1±1.7	9.2±0.9

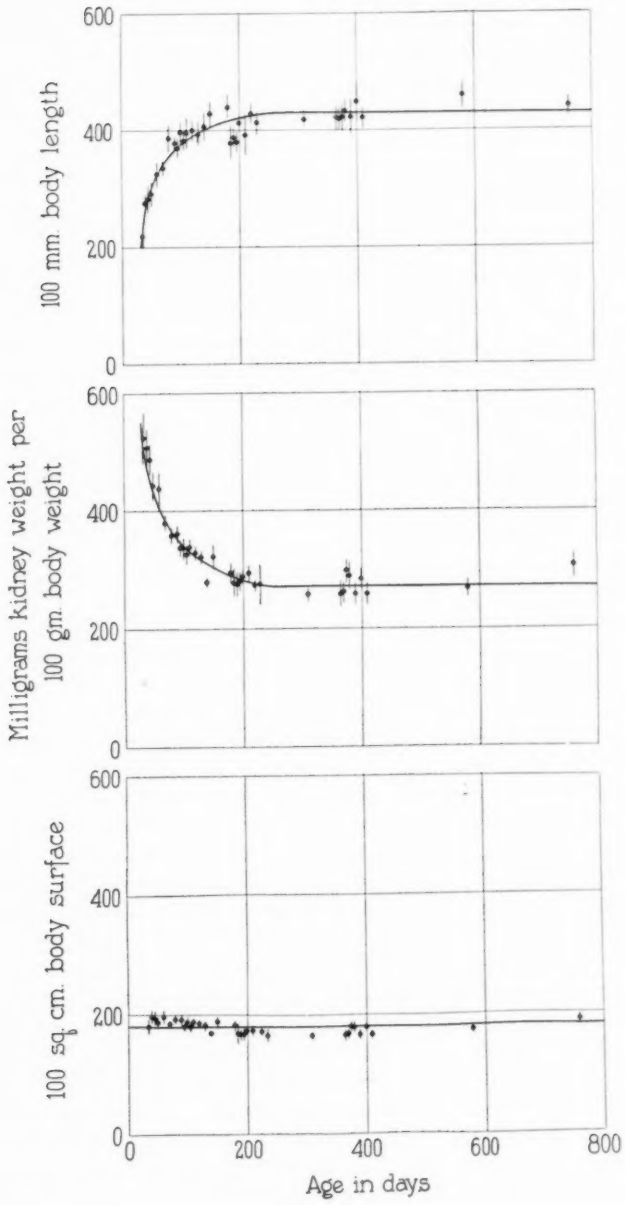
Milligrams kidney weight per
100 mm. body length
100 gm. body weight
100 sq. cm. body surface
600
400
200
0
0
200
400
600
800
Age in days

Fig. 1

a lower percentage increase with age than the increase in the kidney weight in relation to its initial weight, the latter falling between the other two. The kidney weight-age relationships in reference to both body length and body weight are those which might be expected from Donaldson's formulaeic table (1924).

The relation of kidney weight to body surface differs from its relation to the other two measurements (body length and weight) of body size in that it is practically the same at all ages. This is particularly interesting in view of the close relation which has been shown between kidney weight and body surface in the rabbit at all ages by Taylor, Drury and Addis (1923) and in the dog by Stewart (1921). It indicates that, unlike the increases in body weight and length, the percentage increase in body surface with age is of the same order of magnitude as the percentage increase in kidney weight.

It might seem that in this relation between kidney weight and body surface which is constant at all ages we had discovered a justification for dispensing with the selection for experiment of rats of the same age. This however is far from being the case, for we have found (MacKay, MacKay and Addis, 1927a) that the quantative effect of factors which alter the relationship between kidney weight and body surface is very different in young rats than in older animals. Age appears to be one of the primary conditions which determine the growth reactions of the kidney, so that it is particularly necessary in experimental work to group the animals first of all by age. This of course still leaves some variability of body size which calls for another grouping on the basis of size. The only question is whether body weight, body length or body surface should be used as the measure of body size. The constancy of the body surface-kidney weight ratio which we have shown exists for all ages, whenever the conditions are uniform, is an advantage which weighs heavily in favor of the selection of body surface. But in addition we find that the kidney weight bears a less variable relation to body surface than to either body weight or body length. In table 1 the coefficient of variation has been determined for the three groups of body size-kidney weight ratios at each age. Out of 34 age groups the variability is least in 4 for the body weight ratios, and in 11 for the body length ratios while in 17 of the age groups the body surface ratios are the least variable. In the other two the variability is the same for the body length as for the body surface ratios. No ratio shows the most constancy at any particular age. The average coefficients of variation for all age groups show further that of the three indices of body size which have been used the kidney weight bears the most constant relationship to body surface. The average coefficients of variation are for body weight ratios 8.4, for body length ratios 7.7, and for body surface ratios 7.0. Body surface is then the best body size reference standard for comparing the kidney weights of different rats of the same age.

SUMMARY

1. The relation at different ages of kidney weight to three measurements of body size,—body weight, body length and body surface—has been determined in the male albino rat. In relation to body weight kidney weight decreases with increasing age, reaching a minimum value at about 200 days of age while in relation to body length it increases with age attaining a maximum value at about the same age. On the other hand the kidney weight has practically the same relation to body surface at all ages.

2. The kidney weight has a less variable relationship to body surface than to either body weight or body length.

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FACTORS WHICH DETERMINE RENAL WEIGHT[1]

III. Sex

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The female of the albino rat, as in other species, has at a given age considerably more adipose tissue than a male rat of the same age. It would seem then that the weight of renal substance per unit of body size would be less in the female because of the preponderance of inactive body tissue in this sex. However in the observations of the relation of kidney weight to body size in the albino rat which have been recorded (Donaldson, 1924) in the literature no sex difference has been noted. From the data to be reported here a difference in this relationship for the two sexes has been found to exist. In the preceding paper of this series the relation of kidney weight to body size was determined for male animals of different ages. The same relationships have been determined for females and are compared in this paper with the results obtained from the male groups of similar ages.

The data were all obtained from female rats of exact ages by methods which have been described (MacKay and MacKay, 1927). A comparison was made of the kidney weight of every rat at each age in relation to the amount of kidney tissue per unit of the body length, weight and surface. The results are given in table 1. In this table the mean ratios of the kidney weight to the various standards at each age have been summarized with their statistical significance. These means have been plotted against age in figure 1. Each mean has been fitted with wings on each side of the point which cover a range of four times the probable error.

The result gives in a general way relationships corresponding to those found in the case of males. Kidney weight in relation to body weight decreases with increasing age and reaches a minimum value at about 150 days of age. In relation to body length the weight of the kidneys increases with age, reaching a maximum value at about the same time. In both instances the period of 150 days required for the relationships to attain a constant value in the female animals is considerably less than that found for the male rats, which was close to 200 days. That a longer time would

[1] This work was aided by the Wellington Gregg Fund for the Investigation of Bright's disease.

TABLE 1

TABLE 1

AGE	NUMBER OF RATS IN GROUP	AGE GROUP MEANS				KIDNEY WEIGHT PER								
						100 gm. body weight			100 mm. body length			100 sq. cm. body surface		
		Kidney weight	Body weight	Body length	Body surface	Mean M	Standard deviation σ	Coefficient of variation v	Mean M	Standard deviation σ	Coefficient of variation v	Mean M	Standard deviation σ	Coefficient of variation v
days		*mgm.*	*grams*	*mm.*	*sq. cm.*	*mgm.*	*mgm.*		*mgm.*	*mgm.*		*mgm.*	*mgm.*	
35	16	300	57	138	169	531±5.3	31.7±3.8	6.0±0.7	218±2.6	15.2±1.8	7.0±0.8	178±1.3	7.7±0.9	4.3±0.5
40	18	346	77	148	206	448±6.2	26.5±3.0	5.9±0.7	234±2.6	16.3±1.8	7.0±0.8	168±1.3	7.9±0.9	1.7±0.2
45	18	396	85	154	218	470±9.5	60.0±6.7	12.8±1.4	257±4.2	26.2±2.9	10.2±1.1	182±3.1	19.5±2.2	10.7±1.2
50	18	436	101	162	245	434±5.0	31.3±3.5	7.2±0.8	269±2.6	16.1±1.8	6.0±0.7	178±1.6	10.3±1.2	5.8±0.7
60	18	503	135	180	298	374±4.1	26.5±3.0	7.1±0.8	281±3.2	20.0±2.3	7.1±0.8	168±1.7	10.8±1.2	6.4±0.7
70	25	447	130	177	292	346±3.0	21.9±2.1	6.3±0.6	252±1.8	13.5±1.3	5.4±0.5	154±0.9	6.4±0.6	4.2±0.4
80	18	536	169	187	347	318±3.0	19.0±2.6	6.0±0.7	286±2.2	13.9±1.6	4.6±0.6	155±1.1	6.9±0.8	4.5±0.5
90	18	575	199	195	385	292±3.4	21.4±2.4	7.3±0.8	294±3.1	19.2±2.2	6.5±0.7	141±2.1	13.2±1.5	9.4±1.1
95	12	564	178	193	359	317±6.2	23.0±3.2	7.3±1.0	293±3.9	19.8±2.7	6.8±0.9	157±1.8	9.0±1.2	5.7±0.8
100	12	575	179	193	360	323±4.2	21.6±3.0	6.7±0.9	298±5.8	17.2±2.4	5.8±0.8	160±1.8	9.0±1.2	5.6±0.8
105	12	572	177	194	358	325±6.3	29.7±4.1	9.1±1.3	296±2.8	14.3±2.0	4.8±0.7	161±2.1	10.9±1.5	6.8±0.9
110	12	529	176	191	356	301±2.1	10.9±1.5	3.6±0.5	276±4.1	20.9±2.9	7.6±1.0	143±1.5	7.6±1.0	5.3±0.7
120	12	550	184	192	367	300±4.1	21.0±2.9	7.0±1.0	286±4.2	21.7±3.0	7.6±1.2	150±1.9	9.6±1.3	6.4±0.9
130	28	510	173	190	352	296±2.4	18.9±4.7	6.4±0.6	269±2.3	18.4±1.7	6.8±0.6	145±0.9	7.3±0.6	5.0±0.5
140	12	549	200	199	388	283±4.3	22.0±3.0	7.8±1.1	275±3.7	18.9±2.6	6.9±0.9	141±1.7	8.7±1.2	6.2±0.9
180	11	628	221	205	415	284±3.4	16.9±2.4	5.9±0.9	307±5.7	27.9±4.0	9.1±1.8	151±1.7	8.3±1.2	5.5±0.8
185	11	560	200	197	388	281±4.3	21.2±3.1	7.5±1.1	285±4.2	20.5±3.0	7.2±1.0	145±1.6	8.0±1.2	5.5±0.8
190	11	541	198	199	385	275±5.5	26.8±3.9	9.8±1.4	272±4.0	19.6±2.8	7.2±1.0	141±2.4	11.8±1.7	8.4±1.2
195	21	596	205	201	394	293±2.8	19.2±2.0	6.6±0.7	297±3.2	21.8±2.3	7.3±0.8	152±1.3	9.1±1.0	6.0±0.6
200	13	593	205	202	395	290±3.1	11.1±1.5	3.8±0.5	293±3.6	19.4±2.6	6.6±0.9	150±0.7	3.9±0.5	2.6±0.3
210	11	581	207	199	398	282±4.3	21.0±3.0	7.4±1.1	292±4.0	19.5±2.8	6.7±1.0	146±2.6	8.5±1.2	5.8±0.8
220	19	606	226	204	421	270±3.3	21.5±2.4	8.0±0.9	282±4.0	25.8±2.8	9.2±1.0	144±2.2	9.7±1.1	6.7±0.7
230	12	590	217	201	410	271±3.7	19.2±2.6	7.1±1.0	294±5.1	26.1±3.6	8.9±1.2	144±2.8	10.7±1.5	7.4±1.0
400	24	641	220	203	414	291±2.8	17.2±1.7	5.9±0.6	316±2.5	17.9±1.7	5.7±0.6	155±3.4	7.9±0.8	5.1±0.5

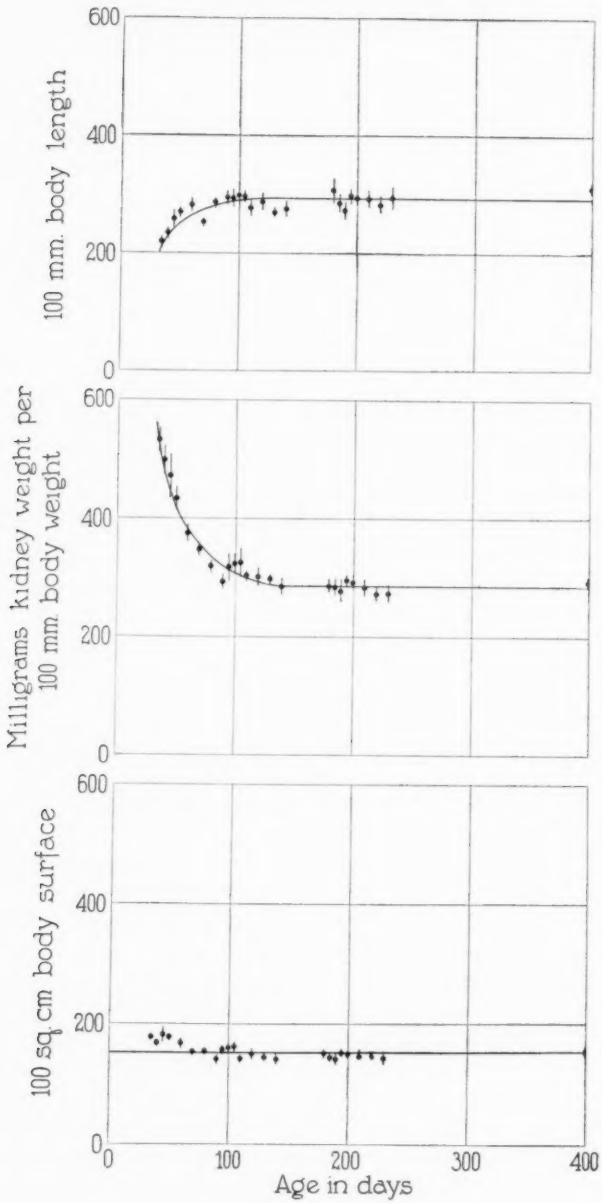

600
400
200
0
100 mm. body length
600
400
200
0
Milligrams kidney weight per 100 mm. body weight
600
400
200
0
100 sq. cm body surface
0
100
200
300
400
Age in days

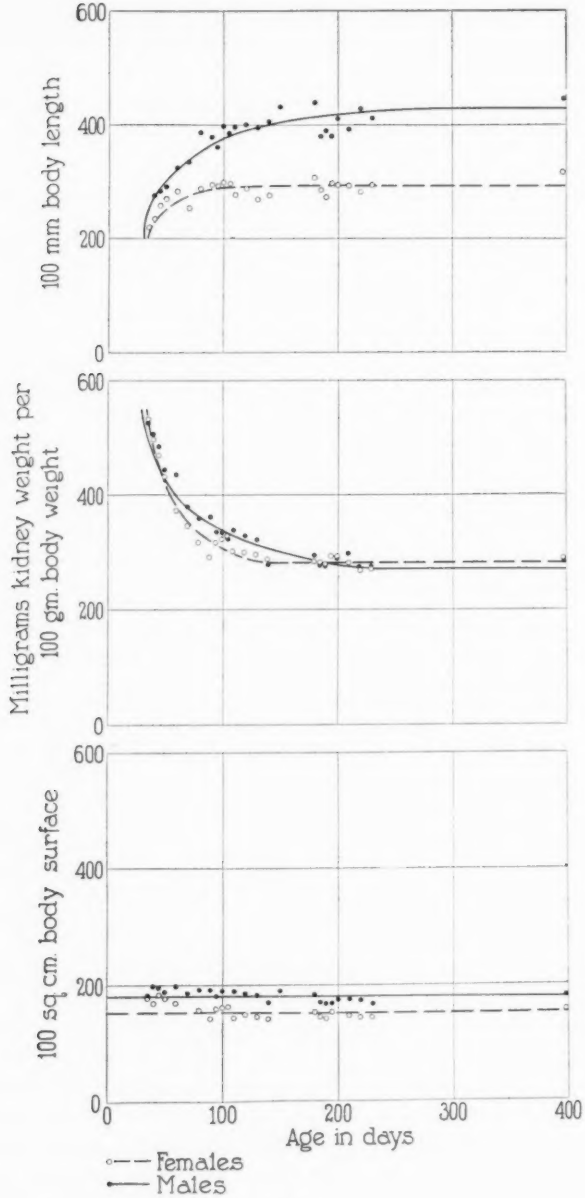
Fig. 1

be required for the kidney weight, body length and weight relationships to become constant in males might be expected because, of the two sexes, the females reach maturity much earlier. The relation of kidney weight to body surface is similar to that found for the male groups in that it is practically the same at all ages.

TABLE 2

DAYS OF AGE	KIDNEY WEIGHT PER											
	100 grams body weight				100 mm. body length				100 sq. cm. body surface			
	Means		Difference		Means		Difference		Means		Difference	
	Males	Females	Actual	Probable	Males	Females	Actual	Probable	Males	Females	Actual	Probable
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
35	524	531	+7	±12.0	219	218	−1	±5.3	180	178	−2	±3.6
40	506	448	−58	±10.4	276	234	−42	±4.6	197	168	−29	±2.9
45	486	470	−16	±11.5	283	257	−26	±7.1	195	182	−13	±4.1
50	444	434	−10	±7.8	291	269	−22	±5.9	187	178	−9	±2.6
60	437	374	−63	±8.5	325	281	−44	±6.5	197	168	−29	±3.8
70	380	346	−34	±4.2	335	252	−83	±3.2	185	154	−30	+1.6
80	358	318	−40	±4.5	386	286	−100	±6.5	192	155	−37	±2.1
90	362	292	−70	±5.3	378	294	−84	±4.5	191	141	−50	±3.0
95	337	317	−20	±7.1	360	293	−67	±5.8	180	157	−23	±2.5
100	336	323	−13	±6.2	397	298	−99	±7.3	187	160	−27	±2.9
105	325	325	0	±7.6	384	296	−88	±5.8	180	161	−19	±2.8
110	339	301	−38	±4.3	396	276	−120	±7.8	187	143	−44	±2.3
120	328	300	−28	±5.1	400	286	−114	±6.2	185	150	−35	±2.6
130	321	296	−25	±4.2	394	269	−125	±5.8	181	145	−36	±2.0
140	279	283	−4	±4.7	406	275	−131	±7.3	169	141	−28	±1.7
180	295	284	−11	±5.5	439	307	−132	±8.1	182	151	−31	±2.9
185	276	281	+5	±7.5	379	285	−94	±8.1	168	145	−23	±4.6
190	275	275	0	±7.4	388	272	−116	±5.7	166	141	−25	±3.1
195	280	293	+13	±5.0	380	297	−83	±5.5	167	152	−15	±2.8
200	288	290	+2	±4.2	412	293	−119	±4.3	174	150	−24	±1.4
210	294	282	−12	±5.4	392	292	−100	±9.6	174	146	−28	±3.3
220	274	270	−4	±4.0	428	282	−146	±5.9	172	144	−28	±2.6
230	275	271	−4	±9.3	412	294	−118	±7.1	165	144	−21	±4.0
400	283	291	+8	±5.0	448	316	−32	±8.9	178	155	−23	±4.1

With male rats body surface was decided upon as the best body size reference standard for comparing the kidney weights of different rats of the same age. This was true not only because the relation between kidney weight and body surface was the same at all ages but because the kidney weight had a less variable relationship to body surface than to either body weight or body length. The same things are true for female rats. In table 1 the coefficient of variation has been determined for the three groups


600
400
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100 mm body length
600
400
200
0
Milligrams kidney weight per 100 gm. body weight
600
400
200
0
100 sq. cm. body surface
0
100
200
300
400
Age in days
Females
Males

Fig. 2

of body size-kidney weight ratios at each age. Out of 24 age groups the variability is least in 1 for the body weight ratios, and in 4 for the body length ratios while in 19 of the age groups the body surface ratios are the least variable. The average coefficients of variation for all age groups show further that of the three indices of body size the kidney weight bears the most constant relationship to body surface. The figures for the average coefficients of variation are for body weight ratios 7.02, for body length ratios 7.00, and for body surface ratios 5.46.

In figure 2 the relation of kidney weight to body weight, length and surface for female rats have been compared to the same relationships for male rats at corresponding ages. The significance of the differences which exist between the ratios at each age is given in table 2 where the probable differences between the age group means have been compared with the actual differences. Only when the actual difference is four times greater than the probable difference is the difference between a pair of ratios significant. Accordingly, it is found that there is definitely less renal substance per unit of body length in the female than in the male animals. The difference is present at all times but is greater the older the rats until they are 100 or more days of age. This sex difference is not so striking in the relation of the kidney weight to body weight, although out of the 24 age groups in which the comparison has been made the ratio is less for the females in 17 and in 8 of these the difference is significant.

Since body surface was decided upon as the best body size reference standard, sex difference in the relation of kidney weight to this factor is especially important. In every age group the kidney weight: body surface ratio was considerably less for the female rats and in all but one group this difference has a decided significance. Sex then, like age, is one of the primary conditions which determine the size of the kidney so that it is absolutely necessary in experiments concerned with renal weight to group animals not only by age but in addition by sex. Further evidence in support of the necessity of grouping by sex is found in the different growth reactions to experimental stimuli of the male and female kidneys. In experiments to be reported later it will be shown that the size of the kidneys in female rats is much less easily altered by changes in the environment than in the case of male rats.

SUMMARY

The female rat has been shown to have less renal tissue per unit of body size than the male rat.

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THE EFFECTS OF HYSTERECTOMY ON THE SYSTEM OF SEX ORGANS AND ON THE PERIODICITY OF THE SEXUAL CYCLE IN THE GUINEA PIG

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In the course of our earlier investigations concerning the mechanism of the sexual cycle and the production of placentomata, we made some observations relating to the prolongation of the life of the corpus luteum and the delay in ovulation resulting therefrom (1). Subsequently we carried out a series of investigations concerning the effects of complete and incomplete hysterectomy on the life and function of the corpus luteum in the guinea pig and demonstrated among other facts a quantitative relation between the amount of uterus extirpated and the effects on the corpus luteum and the sexual cycle (2). We also found that if we extirpate the uterus of immature guinea pigs a new ovulation occurs, thus proving that it is not the extirpation of the uterus as such which prevents ovulation, but the persistence and function of the corpus luteum. Since then we have continued our experiments and confirmed and extended the results previously obtained, and inasmuch as the factors entering our experiments concern the essential conditions underlying the mechanism of the sexual cycle, and as the conclusions of several recent authors (Frank, Allen) in regard to the function of the follicular hormone are at variance with the results of these as well as of our preceding experiments and conclusions, a fuller presentation and discussion of our findings seems to be desirable.

We shall first describe the changes produced by hysterectomy in the later stages following the operation and shall then discuss the conditions found in the earlier stages.

1. *The generative system in guinea pigs 40 to 46 days after hysterectomy.* The hysterectomy was usually complete or almost complete as far as the uterine horn was concerned, but the cervix or at least a part of the cervix was usually left. However, in some cases a small part of the upper end of a uterine horn remained which then had the tendency to close through apposition of the cut ends of the uterine remnant and to become somewhat dilated. Small placentomata could develop in these upper remnants of uterine horns, provided the operation had been carried out at the time when

the uterine mucosa was sensitized. Frequently small parts of the lower ends of the uterine horns were also left. The hysterectomy was as a rule carried out three to five days following oestrus. In animals thus operated on there occasionally occurred slight tears in the vagina, but usually no real opening of the latter was observed. Thus in one case such a tear occurred thirteen days after operation and eighteen days after the preceding heat; in another case the tear appeared twenty-two days after operation and twenty-five days after heat.

At autopsy new corpora lutea were not visible in the ovaries; in case there was an adhesion between the lower end of the uterine horns and the intestines, a necrotic nodule or in some instances also a living placentomata could be seen at the place of adhesion. The vagina, which was closed, contained occasionally a mucoid material. The mammary glands were large. The microscopic examination of the ovaries showed the presence of large, well-preserved corpora lutea. The lutein cells were large and finely vacuolar; their nuclei did not contain a large amount of chromatin and were relatively pale. There were visible within these organs a number of vessels with thick walls. These corpora lutea corresponded in every respect—in size, character of vessels and lutein cells—to the corpora lutea of pregnancy; they were much larger than the corpora lutea of the ordinary cycle. In one animal some collections of lymphocytes were seen around vessels in the corpus luteum. In addition to such corpora lutea there were found atretic yellow bodies,—the remnants of old degenerated corpora lutea,—and furthermore follicles of all sizes, well-preserved ones as well as others in the process of granulosa degeneration, and of connective tissue atresia in its various stages. There were likewise present, in the majority of cases, good mature follicles as well as mature follicles beginning to degenerate. The later stages of degeneration of the follicles, in which the thecae internae were rather prominent, were also noticeable. The ovaries thus resembled very closely those observed in the middle of pregnancy as far as the character of the corpora lutea, of the atretic yellow bodies and the state of the follicles are concerned. In particular we found mature follicles which, however, owing to the presence of the corpora lutea, did not rupture, but which underwent degeneration. Thus a further proof is provided of the fact, formerly established by us, that corpora lutea in the guinea pig do not inhibit maturation of the follicles, but do prevent ovulation.

The vagina is covered by one layer of cylindrical epithelium. These cells are very finely or coarsely vacuolar and may contain much mucus which causes them to take on a bluish color if stained with haematoxylin. The amount of mucus present varies, however, in different cases and also in different places; it seems to be especially great near the cervix. The nuclei of the epithelial cells are situated in the interior and not at the base

of the cells. In some specimens there is visible, in certain places, a layer of flat or very low cuboidal cells below the cylindrical layer. When the epithelium is injured these flat cells may serve as the covering of the vaginal wall. Such an injury to the epithelium may be caused by masses of polynuclear leucocytes, which quite commonly, but not always, migrate through the wall of the vagina into its lumen. They often form small abscess-like collections in the mucosa. There are also occasionally some small collections of lymphocytes in the mucosa. In no case was any proliferation seen in the vaginal epithelium. The connective tissue underneath the vaginal epithelium is fibrous. On the whole the vagina in hysterectomized animals closely resembles the vagina in pregnant animals. The cervix, part of which is very papillary in structure, is covered by one layer of epithelial cylindrical cells. In the recesses between the papillae the epithelial cells are more regularly cylindrical, with nuclei situated toward the base of the cells, while toward the top of the papillae there are more irregularly-formed vacuolar epithelial cells, with nuclei located higher up in the cell. The epithelium of the cervix may also take on a bluish stain owing to the mucus it contains, but in other cases the bluish tint is lacking. The connective tissue in the cervix is less fibrous than in the vagina; especially at its junction with the uterus and at the top of the papillae it is more cellular than at the base of the papillae where it is more fibrous. Here, again, may be found small collections of lymphocytes in the mucosa underneath the epithelium. Polymorphonuclear leucocytes may here also migrate into the lumen of the organ where they are sometimes found in considerable numbers. Toward the uterus, glands appear in the mucosa and the papillary formation disappears; the cylindrical surface epithelium becomes lower and more solid, but occasionally islands of the cylindrical vacuolar vaginal epithelium may be found in the uterine epithelium. In the surface epithelium of the uterus some mitoses can usually be seen, while they are ordinarily lacking in the uterine glands; at times some mitoses may also be observed at the borderline between cervix and uterus. In some cases a few lymphocytes and polymorphonuclear leucocytes may migrate through the uterine wall into the lumen of this organ, but in other cases they are absent. The connective tissue underneath the epithelium is rich in cells as compared to the vaginal connective tissue, but at the same time it is finely fibrillar; the nuclei of the connective tissue cells are small and do not show any proliferation.

While the papillary formations characteristic of the cervix are generally absent in the remnants of the uterine horns, still, owing to the conditions created by the hysterectomy, some blunt papillae may develop here.

The mammary glands are relatively large, consisting ordinarily of lobules, each containing many acini. The acinus cells are well developed and usually a number of mitoses are found in the parenchyma. The stroma con-

tains as a rule some eosinophiles, lymphocytes and well formed connective tissue cells. In the latter mitoses may also occur, but they are rare on the whole.

2. *The generative system in guinea pigs 50 to 100 days following hysterectomy.* During the period from fifty to one hundred days following hysterectomy, the condition of the generative system remained about the same as we found it at forty days. In the ovaries the corpora lutea remained large and well preserved, except that in certain less well nourished parts vacuolization was occasionally noticeable in their peripheral parts; mitoses were seen in some lutein cells even as late as ninety-seven days following extirpation of the uterus. Also some small collections of lymphocytes were occasionally found in some corpora lutea. The follicles underwent the usual cyclic changes. Follicles matured continually and then degeneration set in without ovulation having occurred. Degenerating corpora lutea were not seen, but in some cases old yellow-fibrous vacuolar bodies were found in the ovaries, while in other animals they were lacking. They were probably the remnants of old corpora lutea belonging to cycles preceding the extirpation of the uterus. As late as fifty-four days after heat and fifty-one days following hysterectomy such remnants of old, retrogressing corpora lutea may be lacking, a finding which may serve as a further proof that in these cases no ovulation had taken place following the extirpation of the uterus. In one case we found in an ovary of an animal, which had been hysterectomized ninety-five days previously, a syncytioma which was in process of retrogression, some hemorrhage having taken place in the center of this structure. Another peculiarity was observed in another case examined ninety-five days following hysterectomy; here one ovary was unusually small, while the other ovary behaved typically as far as the character of the corpora lutea and mature follicles was concerned. Toward the surface of this latter ovary there was some hyperemia around the mature follicles, and in the small ovary there were a number of follicles each one with a hypertrophic, very hyperemic theca interna. The enlarged theca cells were surrounded by dilated capillaries. Hemorrhages, which had occurred in some hyperemic places, had partly detached the granulosa of these follicles from their theca interna, the granulosa cells becoming partly shrunken as a result of the detachment. As far as we could make out, no rupture had taken place in this animal but some small follicles had been destroyed as a result of the hyperemia.

In vagina and cervix no noticeable change takes place during this period. As usual a part of the cervix is papillary, and the papillae are lined with one layer of cylindrical cells, which are on the whole regular in shape and arrangement. Similarly the vagina is lined with one layer of cylindrical cells, which are more vacuolar and irregular than those covering the cervix. There may be one layer of very low cuboidal or flat cells underneath the

epithelium of the vagina and the transitional area between cervix and vagina; in other cases this layer seems to be lacking. It is difficult to decide in some cases whether we have to deal in this layer with connective tissue cells or with low and flat epithelium. Again some polymorphonuclear leucocytes may migrate through the wall of the vagina or cervix and collect in the lumen of these structures, or occasionally they may make some abscess-like formations within the epithelium. Also some lymphocytes may be found in the wall of the vagina below the epithelium.

In the mammary gland proliferative activity continued during this period; in almost all cases mitoses were seen in gland cells and the glands were large and consisted of many acini. In the stroma lymphocytes and eosinophiles were found. In one case, (guinea pig 1461), we observed milk secretion in the mammary gland which had proliferated. In this animal the uterus had been extirpated five days following ovulation. Fifty-three days after this operation, the ovaries were extirpated and two days later vagina and mammary gland were taken out for examination. They behaved in the typical manner; there were found a persistent large corpus luteum and a vacuolar vaginal epithelium; the mammary gland had proliferated, and in addition secondary changes had taken place which led to milk formation. Microscopically in some of the acini the epithelium contained droplets as an indication of this milk secretion. Whether or not the extirpation of the ovaries was responsible for this result, will have to be determined in further experiments. In general, however, the mammary gland of hysterectomized guinea pigs undergoes proliferation but does not secrete milk.

3. *Does ovulation occur after extirpation of the uterus?* If the uterus is extirpated a few days following ovulation, ovulation does not usually occur within three months following the operation. In rare cases an ovulation may perhaps take place. The vagina no longer fully opens and on examining the ovaries of such guinea pigs microscopically we have not as a rule found an indication of a recent ovulation; on the contrary we usually observed large, well-developed, old corpora lutea, and, in addition, in many cases some small remnants of corpora lutea belonging to pre-operative cycles; these remnants had the form of fibrous yellow bodies. However, in one guinea pig in which examination took place eighty-six days after extirpation of the uterus, we found in the ovaries old corpora lutea with thick-walled vessels in an early stage of degeneration, and, besides, new corpora lutea with connective tissue in the center. The follicles were well preserved and in normal development; one follicle was mature. Granulosa degeneration was just beginning; the mammary gland was proliferating. In this case ovulation had taken place about seven to eight days previous to the examination.

In another animal, which was kept for more than six months following

extirpation of the uterus, the vagina opened again four and five months after the operation. In this case the examination of the ovaries was unfortunately incomplete. In another case, to which we shall refer shortly, ovulation had apparently taken place at an earlier period.

4. *The generative system in guinea pigs within the first five weeks following hysterectomy.* Within the first few weeks, the corpora lutea develop normally, mitoses appear in the lutein cells and the corpora lutea following extirpation of the uterus gradually reach a size similar to that of corpora lutea in pregnant animals. The walls of the blood vessels increase in thickness. Remnants of retrogressed corpora lutea from the cycles preceding the last one are still visible. Such remnants of old corpora lutea may also be found during the latter part of a normal pregnancy. In a few cases we found in addition to the typical large, well preserved corpora lutea, characteristic of hysterectomized animals, which were present in one of the two ovaries, in the second ovary one or several vacuolar corpora lutea which showed a beginning degeneration. We may therefore conclude that in some cases not all of the corpora lutea survive, but that one may retrogress and may in the course of time become a vacuolar yellow fibrous body, while the others remain alive and function and enlarge.

There may thus be two kinds of atretic yellow bodies in the ovaries of hysterectomized guinea pigs, namely, 1, the remnants of degenerated corpora lutea of former cycles, and 2, the remnant of a corpora lutea which retrogressed in one ovary, while the corpora lutea of the other ovary remained well preserved. Such retrogression of the corpora lutea in one ovary, in contradistinction to the preservation of those in the other ovary, may become noticeable in the third or fourth week following the preceding heat. This occurrence is not limited to guinea pigs in which a complete or almost complete hysterectomy has been carried out, but it may likewise be found in animals in which a partial extirpation of the uterus has been done or in which merely incisions have been made into the uterus. In all these cases the life of the corpora lutea may be prolonged, but in the latter two experiments this prolongation is very much more limited than when the first experiment is made. We may then notice in animals in which only incisions have been made into the wall of the uterus that the corpora lutea in both ovaries begin to retrogress after some time, but that the retrogression has progressed further in the corpora lutea of one ovary than in those of the other ovary. As long as at least one well preserved corpus luteum is left, the typical effects of this organ are noticeable.

While, as stated, we usually found during this period well preserved large corpora lutea in the ovaries of guinea pigs whose uterus had been completely or almost completely extirpated within the first week following ovulation, there is the possibility that exceptionally a new ovulation may occur under those conditions. One case of this kind we have thus far

encountered (guinea pig 1313), in which six and one-half days following heat both ovaries had been almost completely removed and in which sixteen days later, twenty-two to twenty-three days following ovulation, in addition to a corpus luteum in an early process of degeneration, a new corpus luteum was found; the latter had still a large cavity. The large and medium sized follicles were all in early stages of atresia. Ovulation had in these cases taken place about a day previously.

In the usual cases after complete or almost complete hysterectomy the corpora lutea remain preserved and in addition mature follicles develop in these ovaries in the typical manner; but, instead of rupturing, they then degenerate because of the function of the corpus luteum and new mature follicles soon take the place of the degenerated ones. At an early stage the vagina assumes the characteristic structure which we found in later periods after hysterectomy. The vaginal epithelium consists of one layer of vacuolar cylindrical cells. Underneath the latter there may be seen, at least in certain places, a layer of flat or low cuboidal cells. The cervix behaves in the same way as in the later stages following the operation. The mammary gland is generally found in a proliferating state. If small remnants of the uterus were left at the upper or lower end, placentomata may develop near the cut surface. In one case, (guinea pig 1315), we succeeded in excising all three corpora lutea, which had formed, simultaneously with the almost complete extirpation of the uterus five days following oestrus. Fifteen days later the small uterine remnants presented a typical predeciduomatous mucosa, thus indicating that about seven to eight days previously a new ovulation had occurred. In the ovaries newly formed corpora lutea were present, the age of which corresponded to the time of ovulation. We may therefore conclude that in this case thirteen days following the last period of heat a new ovulation occurred, notwithstanding the preceding hysterectomy; this effect is in agreement with our previous findings relative to the acceleration of ovulation following a complete extirpation of existing corpora lutea. This observation suggests furthermore that it is not the hysterectomy as such, but the persistence of the corpora lutea following hysterectomy which prevents a new ovulation. Such a conclusion is confirmed by the following experiments in which hysterectomy was carried out in immature guinea pigs.

5. *The effects of hysterectomy in immature guinea pigs.* As the outstanding conclusion in these experiments we may state that ovulation can be readily observed to take place after complete extirpation of the uterus, provided hysterectomy is carried out in immature guinea pigs. In this case ovulation takes place as soon as the animal has recovered from the effects of the operation and the follicles have reached maturity. It occurred in our experiments as early as nine and as late as twenty-nine days

following hysterectomy. We may cite a few examples of the results of hysterectomy in immature guinea pigs.

1. Guinea pig 490, in which the weight at time of hysterectomy was 200 grams; ovulation had not yet taken place. Fifteen days following operation the vagina was partly open; on the following, the sixteenth day after operation, it was fully open and examination was made. Weight at that time was 260 grams. The ovaries showed well-preserved follicles of all sizes; other follicles were in granulosa degeneration and in early stages of connective tissue atresia. There were present two large mature follicles in a state preceding rupture; but ovulation had not yet actually occurred. The vagina showed active proliferation; it consisted of well developed squamous epithelium which was covered by a layer of keratin, to which was attached the cast off and raised up cylindrical epithelium. A number of mitoses were seen in the basal layer of the squamous epithelium. Toward the cervix the latter gradually decreased in thickness and the formation of keratin ceased, while fine papillae developed. In the remnants of the uterus there was cylindrical surface epithelium; the short glands entering the latter showed epithelium, which was in mitotic activity. Also in the connective tissue around the glands some mitoses were seen. The mammary gland consisted of some large ducts covered by rather high epithelium, likewise in mitotic proliferation.

2. Similar were the findings in guinea pig 491 (weight 225–280 grams). In this case the vagina opened nineteen days after hysterectomy which had been carried out in the as yet immature animal. Examination of the ovaries revealed two follicles which had ruptured a very short time previously; their granulosa had just begun to be transformed into lutein tissue.

The epithelium of the vagina had been changed through mitotic activity into squamous epithelium, which was covered by keratin. The mammary gland showed a few branching ducts with mitoses in the epithelium, but its stroma was still densely fibrillar.

3. In guinea pig 1515 (115–545 grams), heat occurred twenty-seven days following hysterectomy. Following this period of heat the vagina remained closed for the following fifty-eight days, when, eighty-five days after operation, the organs were removed for examination. The ovaries showed large, good corpora lutea with mitoses in the lutein cells and quite mature follicles. There was found in addition a very small, yellow vacuolar body. It probably represents a corpus luteum which retrogressed earlier than the others. In accordance with expectation, the vagina was lined with vacuolar cylindrical epithelium. The mammary gland consisted of many acini which did not show mitoses.

4. In another animal, guinea pig 1511 (250–417 grams), heat was ob-

served twenty-nine days following hysterectomy. The vagina did not open in the subsequent forty-nine days. When the organs were taken out for examination, the ovaries showed two large well developed corpora lutea without signs of degeneration; the walls of the vessels were thick. No retrogressing corpus luteum nor yellow body was visible. There were seen large, well preserved immature, as well as mature, follicles, also follicles in granulosa degeneration; a syncytioma was found in one ovary. The vagina showed the typical single layer of cylindrical vacuolar epithelium. The mammary gland was well developed, consisting of many acini.

These cases are typical of the findings in a number of other similar experiments. The following conclusions may be drawn from these observations: 1. Extirpation of the uterus as such does not prevent ovulation. In an immature guinea pig, ovulation takes place at the time maturity is reached, notwithstanding the complete or almost complete extirpation of the uterus. It is the presence of a functioning corpus luteum which prevents ovulation. After the first ovulation has occurred in the mature guinea pig, a corpus luteum develops and subsequently no ovulation takes place, at least for a long period of time. There is some indication that after five or six months, or in some cases at a somewhat earlier period, the vagina may again open. Whether a regular cycle is subsequently established needs still further investigation. 2. Extirpation of the uterus as such does not lead to a noticeable injury of the vagina. This is shown by the fact that after extirpation of the uterus in an immature guinea pig ovulation occurs, the vagina undergoes the typical proliferative processes, which have already begun in the normal manner before the time of actual ovulation. The ovaries show at this period the usual rapid atresia of all except the smallest follicles. 3. Subsequently, when the corpus luteum has become established and functions, follicles mature constantly, but do not rupture; instead they become atretic.

Under these conditions the vaginal epithelium remains inactive; the epithelial cells maintain their cylindrical vacuolar character and often contain mucus. This condition cannot be due to the inability of the vagina to undergo the typical proliferative changes: the observations, just mentioned, exclude such an interpretation; neither can it be due to the absence of the active follicular substance, because follicles mature constantly under these conditions. There remains only the conclusion that it is the corpus luteum which prevents these changes. In a similar way during pregnancy where also maturation of the follicles, without subsequent rupture, occurs, the vaginal changes do not occur, owing to the function of the corpus luteum. We may therefore conclude that the living and functioning corpus luteum after hysterectomy not only prevents ovulation but also the cyclic proliferative changes in the vagina which normally take

place probably under the influence of a substance given off by follicles. While we have established the fact that in the guinea pig the functioning corpus luteum inhibits ovulation during the ordinary cycle as well as during pregnancy and after hysterectomy, as far as the proliferative changes in the vagina are concerned, our experiments so far prove only that it is the large persistent corpus luteum of hysterectomy and of pregnancy which prevents these growth processes and the production of squamous epithelium. Although the fact that, during the life and functioning of the ordinary cyclic corpus luteum, the vagina does not open and that, during the latter part of the ordinary cycle, the vagina does not yet show transformation into the squamous epithelium, renders it probable that the cyclic corpus luteum also inhibits growth processes in the vagina, still it will be necessary to carry out special experiments in order to answer these questions definitely.

If the uterus is extirpated in immature guinea pigs and corpora lutea develop subsequently which then do not retrogress, yellow vacuolar fibrous bodies are not usually found in the ovary; this is a further indication that, as long as these corpora lutea persist, a new ovulation does not occur. If exceptionally such a yellow body does occur, its function is probably the result of a premature retrogression of one of the corpora lutea developing at the time of the first ovulation.

The ovulation which occurs in an immature guinea pig after hysterectomy can be prevented if we prevent the development of the follicles through undernourishment. In one immature guinea pig (no. 85) this result was obtained accidentally. In this case, in which the examination took place thirty-three days following operation, adhesions between the wound in the uterus and the intestines caused undernourishment. A hypotypical state of the ovaries resulted from this adhesion; follicles failed to mature; ovulation and formation of the corpus luteum did not occur. As a result of this condition the vaginal epithelium remained cylindrical and vacuolar and in a resting state. Lymphocytes and polymorphonuclear leucocytes migrated through it. The mammary gland consisted only of a few resting ducts surrounded by fibrous tissue.

We may then conclude on the basis of these experiments that the extirpation of the uterus as such does not have a direct stimulating effect on the mammary gland.

6. *The effects of incomplete extirpation of the uterus.* After we had established the marked effect which a complete or almost complete hysterectomy has on the sexual cycle, it was of interest to determine how great a portion of the uterus needs to be extirpated in order to obtain these results, and whether the particular part which is extirpated affects the result, and lastly whether an incomplete extirpation, although not fully effective, might still modify the sexual cycle in the same direction in

which a complete hysterectomy affects it. We varied accordingly our experiments in such a way that not only the length but also the part of the uterus which was removed differed in different cases.

1. In several experiments we extirpated, three days after heat, the cervix and the adjoining lower portion of the two uterine horns; approximately perhaps one-fifth or one-sixth of the latter structure was removed. In these cases a new oestrus occurred twenty-one, twenty-seven and, in two animals, thirty days after the preceding heat; or, eighteen, twenty-four and twenty-seven days, respectively, following the operation. The organs were taken out soon after the occurrence of the new heat and showed a structure which corresponded to the phase of the sexual cycle in which the examination took place. In particular we found, in addition to newly formed corpora lutea, the corpora lutea of the preceding cycle in a state of retrogression; the vaginal epithelium had the character of keratinizing squamous epithelium and, likewise, the state of the mammary gland and of the remaining parts of the uterus corresponded to an early stage of the cycle. The lower ends of the upper portions of the uterine horns, which were thus separated from the cervix, were in some cases found closed and the horns themselves dilated through hemorrhagic fluid, which had collected in the uterine cavity probably as the result of the hyperemia occurring in the uterine mucosa at the time of heat.

2. In other experiments, about one-half, or somewhat more, of the upper portions of both uterine horns were extirpated and in the lower portions, which were left in some cases, longitudinal incisions were made in such a way that the greater part of the remaining horns were slit open. If incisions of this kind were made into the uterus, approximately four to seven days following the period of heat, placentomata developed in the upper end of the remaining portions of the uterine horns; if the incisions were made one or two days following oestrus, and the upper ends of the lower remaining portions of the uterine horns were ligated and burned, placentomata did not develop. In general, the onset of the next cycle is distinctly delayed as a result of the incomplete extirpation of the uterus; but an at least partial recovery usually takes place and the subsequent second and third periods of heat occur as a rule with less delay than the first heat following the operation, and they may be observed at approximately the normal time or with only a slight retardation. The normal period between two successive heats in the guinea pig is approximately fifteen to seventeen days.

The following experiments may be cited as examples:

a. In guinea pig 434, two days after heat, a partial extirpation of uterus was carried out; the first heat period following operation occurred twenty-three days after the previous oestrus; the second and third heat periods took place at intervals of about seventeen and eighteen days.

b. In guinea pig 1503, partial extirpation of uterus was made six days following oestrus. Twenty-five days after this heat period a second oestrus occurred.

c. In guinea pig 1508 the operation took place two days following heat. The first cycle following the operation had a duration of thirty-one days and the second a duration of eighteen days.

d. In guinea pig 1510, which was operated on two days after heat, the first cycle lasted thirty days and the second longer than fifteen days.

e. In guinea pig 1512, operated on two days after heat, the first cycle had a duration of twenty-five and the second, nineteen, days. On examination, the various sex organs in the animals just mentioned showed a condition in accordance with the period in the sexual cycle at which they had been taken out for examination. Our observations indicate that in this case also the delay in the occurrence of a new heat is associated with, and probably due to, a somewhat prolonged life and functioning of the corpus luteum; at least in those cases in which the ovaries were examined during the later part of these prolonged cycles, the corpora lutea were found better preserved than they would have been in animals in which the uterus had been intact. Thus we found corpora lutea which were fairly well preserved fifteen, seventeen, twenty-one and thirty-two days after the preceding oestrus, while usually degeneration sets in about thirteen to fourteen days following oestrus.

3. In one case (guinea pig 84), a partial hysterectomy was made out in an immature animal weighing 238 grams. In this case the first ovulation occurred fifteen to sixteen days after operation, the second ovulation about thirty-two days following the first heat period and about two days after this second oestrus the sex organs were examined and found in a condition corresponding to this state of the cycle. One set of very young developing corpora lutea and another set of large old vacuolar, degenerating corpora lutea were found. In this case the partial hysterectomy prolonged, therefore, the duration of the first sexual period considerably, approximately doubling its length.

4. In a fourth series we carried out further control experiments in which, instead of extirpating parts of the uterus, we made merely incisions into the uterus. If these cuts are made about four to seven days following ovulation, placentomata developed. In accordance with our previous findings we observed again that such incisions into the uterine horn prolong somewhat the sexual period. Accordingly they may slightly prolong the life of the corpora lutea and somewhat delay ovulation. In these cases the new ovulation seems on the average to occur at about ninteeen to twenty-three days following the preceding heat period. It is therefore probable that cuts into the uterus delay the ovulation and prolong the life of the corpus luteum on the average a little less than extirpation of the uterine

horns, but inasmuch as in both series there is in individual cases a certain variability as to the degree of delay caused by the experimental interference, some reservation, regarding the relative effects of excision and incision on the life of the corpus luteum and the periodicity of the cycle, is needed at the present time.

In case the life of the corpus luteum was only slightly prolonged through partial hysterectomy, we did not as a rule find the pronounced development of the mammary gland which we observed following complete or almost complete hysterectomy. However, proliferative processes could in certain cases be observed according to the state of the sexual cycle at which the organs were examined.

7. *The effect of the prolongation of life of the corpus luteum on the life and growth of the placentomata.* In those experiments in which either small parts, or somewhat larger parts, of the uterine horns were left and in all those cases in which merely incisions were made into the uterine wall, placentomata could develop in the uterine mucosa, provided the incision had been made at a time when the corpus luteum had had a chance to sensitize the uterine mucosa; such a sensitization is demonstrable usually between the third or fourth to the seventh day following ovulation. These placentomata are especially liable to occur if longitudinal and transverse cuts have been made into the remnant of the uterine horn. In our previous investigations we found that the experimentally produced placentomata have a limited duration of life; they do not usually proliferate later than thirteen days and are generally necrotic fifteen days following the incisions into the uterus. We found furthermore some indications for the conclusion that during pregnancy their life may be somewhat prolonged. We also observed a relation between the duration of life of the placentomata and a new ovulation which occurred following the development of these structures; quite commonly the greater part of the placentomata became necrotic at the time of a new ovulation, presumably owing to the disturbances in circulation at that period (3). In agreement with this latter observation are those experiments in which, after a partial hysterectomy, placentomata developed; these were found necrotic, after a preceding ovulation. In some instances, however, great parts of these new formations may become necrotic in cases in which an ovulation has not yet taken place, but in which the corpus luteum is degenerating, or, we may even find associated a large necrotic placentomata with a living corpus luteum.

We have seen that, after an almost complete extirpation of the uterus, the corpus luteum remains preserved for a long period of time and that, correspondingly, ovulation is prevented. We may therefore expect that the life of placentomata which develop in remnants of the uterine horns may also be prolonged. This is indeed true in some cases. Thus in

guinea pig 418, in which the greater part of the uterus had been extirpated six days following heat, the organs were examined sixty-four days after the preceding oestrus. In this specimen, living and proliferating placentomatous tissue was found at the end of the remnants of the uterus. In another case, (guinea pig 310), growing placentomatous tissue was found forty-one days after extirpation of the uterus and forty-six days after the preceding oestrus. In still another animal there was active proliferation twenty-six days following operation, but usually growth ceased two and one-half to three weeks after operation and at the same time the necrosis progressed. In various experiments the placentomata were found entirely, or at least largely, necrotic four and one-half to six weeks following operation.

Accidental factors determined to some extent the fate of the placentomata. The outer parts which received their nourishment through the peritoneal fluid could be found alive at a time when the less favorably situated parts had perished. An especially unfavorable action on the life of the placentomatous tissue was exerted by adhesions to the large intestines, which occasionally developed. In such cases, as a result of bacterial action, localized necroses and infiltration with polynuclear leucocytes were common. If necrosis occurred as a result of nourishment, which within certain limits was deficient, the cell death was usually preceded by certain abnormal growth processes, as, for instance, development of giant nuclei combined with hyperchromatosis and abnormal increase in size of the whole cell, or by the development of multinucleated giant cells. Growth stimuli, which reached cells living under unfavorable conditions as far as supply of oxygen and other substances are concerned, were responsible for these pathological changes. Gradually these deficiencies in nourishment led to the death of the cells. We find thus that even a prolonged life of the corpus luteum, produced experimentally through an almost complete extirpation of the uterus, cannot insure the continued life and proliferation of the experimentally produced placentomata.

When there is thus some indication that directly or indirectly the corpus luteum may prolong the life of the placentomata, there exists conversely also the possibility that living placentomata may prolong the life of the corpus luteum in certain cases. However that may be, the prolongation of the life of the corpus luteum, following hysterectomy, is independent of the presence of living placentomata; the corpus luteum persists even after necrosis has set in in the placentomata or without the development of the latter structures.

On the basis of these experiments we may draw the following conclusions: 1. If only a very small portion of the uterine horns is left, while the greater part is extirpated, placentomata develop under the conditions stated previously and the life of the corpus luteum is prolonged to the same extent,

or almost to the same extent, as if the extirpation was complete, and the typical effects of the function of the corpus luteum become evident. 2. If a somewhat larger part, perhaps one-third to one-half, of the uterus is left the life of the corpus luteum is prolonged and, as the result of this effect on the corpus luteum, the duration of the sexual period is likewise prolonged, but in both cases the prolongation is much less pronounced than after a more complete extirpation. This conclusion applies in particular to the first sexual period and the first corpus luteum following operation. Subsequent cycles approach more closely the normal conditions. In future experiments it will be necessary to determine more exactly the length of the piece of uterus required in order to avoid the effects of a complete hysterectomy. 3. The results of the experiments are the same, whether the upper or lower portion of the uterus is extirpated in a given guinea pig. The effects of hysterectomy, therefore, do not depend upon the extirpation of a specific part of the uterus or of a specific nervous or vascular structure, but on the quantity of the uterus removed. 4. Mere incisions into the uterus also prolong slightly the life and function of the corpus luteum and the duration of the first sexual period following the operation; but this effect is apparently somewhat less intense than after partial hysterectomy. In this case, also, further comparative experiments are required. 5. The delay in ovulation after complete or partial hysterectomy tends to prolong the life and growth of the placentomata which develop in the uterus under conditions stated previously; however, the growth and life of the placentomata do not necessarily correspond to the duration of life and function of the corpus luteum. Thus the persisting corpus luteum is not able to cause the continued life and growth of the placentomata, although there are some indications that it may prolong both. After the latter have once developed under the influence of the corpus luteum secretion, other conditions, which are, in part at least, variable, enter as factors which influence the further life and growth of the placentomata. 6. After partial hysterectomy, the mammary gland does not undergo the same marked development as after complete or almost complete hysterectomy.

8. *Transplantation of pieces of uterus into hysterectomized guinea pigs.* We have seen that the effects of a complete or almost complete, and of an incomplete hysterectomy, differ quantitatively from each other. It was therefore conceivable that transplantation of pieces of the almost completely extirpated uterus into the subcutaneous tissue of the same animal might take the place of the lacking uterus, and thus make up for the deficiency created, provided the uterus exerts its effects on the corpus luteum through an internal secretion. For this reason we autotransplanted in seven cases at the time of the hysterectomy, pieces of the extirpated uterus into the subcutaneous tissue. We found that these transplants did not

affect the results of the hysterectomy; the corpora lutea remained alive and ovulation was prevented in the same way as in completely hysterectomized animals. The transplantation of pieces of uterus did not therefore change the results of a complete into those of a partial hysterectomy, as far as the effects on the corpus luteum and on the cycle were concerned, notwithstanding the survival of parts of the transplanted uterus and the development of placentomatous tissue in some of the transplants. If the transplantation was carried out one to two days after heat, the transplanted tisuses, including surface epithelium, glands, connective tissue of mucosa and muscle tissue, could remain alive, but no placentomata developed; if the transplantation was carried out five to six days after heat, placentomata usually developed. Parts of the placentomatous tissue could remain alive for as long as almost six weeks after operation. As late as forty-one days following operation, it was still possible to observe some mitotic proliferation in less differentiated small deciduomatous cells, but usually at that time the tissue had differentiated to such an extent that only amitotic, and no mitotic, nuclear divisions were visible. The inability of these transplants to modify the results of hysterectomy does not necessarily exclude the possibility that the uterus gives off an internal secretion, which acts on the ovary and in particular on the corpus luteum. This negative result of the transplantation may be due to the fact that the surviving part of the transplant is relatively small and perhaps does not function satisfactorily. On the other hand, these experiments prove that parts of the developing placentomata may remain alive and sometimes even proliferate during a relatively long period of time.

In five additional experiments we transplanted pieces of uterus of other guinea pigs subcutaneously into the hysterectomized animals at periods varying approximately between seven and ten weeks following the hysterectomy. For this purpose the uterus was extirpated from guinea pigs, which were in a stage of the sexual cycle corresponding to a period of five days following oestrus. The transplanted uterus was therefore sensitized at the time of the operation. In the host there were at this time living corpora lutea present which were much older, as the animals serving as hosts had long since passed the period when normally the corpora lutea sensitize the uterine mucosa. In three of these cases no placentomata developed in the transplant, while in two animals, in which host and donor were nearly related, placentomata did develop.

Again the placentomata found under these conditions, or the surviving transplanted uterine tissues, did not lead, under these conditions, to the preservation of the corpora lutea and to an ovulation in the host animal. They were therefore as ineffective in this respect as the autotransplanted pieces of uterus. We may therefore conclude that transplanted pieces of uterus cannot take the place of the uterine horns acting in their normal situation.

9. *A comparison between the sex organs in hysterectomized and in pregnant guinea pigs.* There is a striking similarity between the generative system in hysterectomized and in pregnant guinea pigs. In the ovaries we find, in both conditions of the animals, corpora lutea, which are much larger than the corpora lutea of the ordinary sexual cycle. The wall of the vessels in the former becomes thick and mitotic figures are seen in the lutein cells. Above all, these corpora lutea persist for a long time. Especially does this hold good in the case of the hysterectomized animals. Here the duration of life of the corpora lutea is much longer than in the pregnant animals, where near the time of birth a degeneration of these organs sets in. The follicles in both hysterectomized and pregnant guinea pigs continue to develop and mature in the normal manner. In the ovaries of both we find, almost continually, new crops of mature follicles springing up; but instead of rupturing they undergo atresia in the same way in one as in the other. Ovulation is therefore suspended in hysterectomized as well as in pregnant guinea pigs. During pregnancy the cyclic changes in the uterus are in abeyance; and this is likewise true in the remnants of the uterine horns in the almost completely hysterectomized guinea pigs. The great atrophy of the uterine mucosa in the pregnant animal is however not noticeable in the uterine remnant near the cervix of hysterectomized guinea pigs. Furthermore, if we call forth in pregnant guinea pigs a new ovulation through extirpation of the corpora lutea, the cyclic changes are lacking in the pregnant guinea pigs, while they take place in a normal manner in the almost completely hysterectomized animals. There is thus found structurally and functionally an atrophy in the uterus of the pregnant animals, which is lacking in the remnant of the uterus of the hysterectomized guinea pigs. The vagina of both the pregnant and hysterectomized guinea pigs is in a resting condition, lined with cylindrical, very vacuolar epithelium in which no growth processes are taking place. This type of epithelial covering apparently does not furnish protection against the penetration of substances from the lumen into the wall of the vagina to the same extent as the well developed stratified epithelium would do, or perhaps substances present in these epithelial cells may attract leucocytes; consequently we often find, in pregnant as well as in hysterectomized guinea pigs, polymorphonuclear leucocytes migrating through the mucosa and they are sometimes so large a number that small abscess-like accumulations of these cells develop in the epithelium of the mucosa.

This complete inactivity and the frequent mucoid changes, which we find in the vaginal epithelium under these conditions, are not due to an injury of the vaginal mucosa resulting from the hysterectomy. If we induce an ovulation in hysterectomized guinea pigs, the normal proliferating activity and the rapid transformation of the cylindrical into the keratinizing squamous epithelium occur in the typical manner in these animals,

notwithstanding the previous extirpation of the uterus. Such an ovulation can, as stated previously, be readily observed after hysterectomy in immature guinea pigs, in which an ovulation has not yet occurred at the time of the operation, and in which therefore a corpus luteum does not yet exert its characteristic influence of suspension of ovulation. Furthermore through extirpation of the corpora lutea, we likewise can call forth a new ovulation in pregnant as well as in hysterectomized guinea pigs, and again we find this condition to be followed by cyclic changes in the uterus, which latter had presumably been preceded by vaginal proliferation. We may conclude that it is merely the persistence of corpora lutea which leads to the suspension of the cyclic changes in the hysterectomized guinea pigs.

However it is not only ovulation which is prevented by the large persistent corpus luteum. In addition this organ also inhibits the proliferation of the vaginal epithelium which in the ordinary cycle has already begun previous to ovulation, at a time when there are large, mature follicles present, or sometimes even without the presence of fully mature follicles. Evidently small amounts of the follicular fluid are constantly absorbed from these follicles, or the active substance may perhaps be secreted directly by the granulosa cells and thus gain access to the circulation and cause the changes in the vaginal mucosa. In addition, there may possibly be other structures in the ovary which give off a similar substance. However, these small amounts, acting constantly, while efficient in the normal guinea pig, at a time when the corpus luteum has begun to degenerate, are inactive when the corpus luteum is functioning. This conclusion applies definitely as far as the large persistent corpus luteum of hysterectomy and of pregnancy is concerned. There are also indications that it also applies to the ordinary cyclic corpus luteum, in which as far as we have observed so far, the vaginal change occurs as soon as the degeneration of the corpus luteum has begun. However we intend to investigate this point still further and at present we do not wish to exclude with certainty the possibility that the proliferation may not begin in certain cases previous to that time. However that may be our experiments concerning the effect of the injections of follicular extract in the guinea pig have shown that the persistent large corpus luteum of hysterectomy and also of pregnancy has a more strongly inhibiting influence on this proliferation than the ordinary cyclic corpus luteum. Thus follicles near maturation, or mature follicles which develop in normal guinea pigs during the cycle, are inactive, because the corpus luteum functions at this time, but they become active as soon as the corpus luteum ceases to give up the substance which in some way neutralizes the follicular substance. For the same reason the vagina is inactive in the hysterectomized and in the pregnant guinea pig, because here also corpora lutea counteract the effects

of the substances given off by the mature follicles. How the vagina would act in the pregnant guinea pig, following an extirpation of the corpora lutea, needs still to be investigated.

In both pregnant and hysterectomized guinea pigs there takes place a very marked development of the mammary gland. Through mitotic growth of the duct epithelium and later also of the acinar epithelium, new acini and lobules of the gland are produced. In all probability these growth processes likewise take place under the influence of the corpora lutea which persist in both states. We must, however, consider the fact that in these two cases there are present in addition to the corpora lutea, mature follicles which constantly develop and then degenerate. As we have previously shown the follicles also give off a substance which stimulates the proliferative activity of the mammary gland. It is therefore possible that this latter substance may contribute to the growth of the mammary gland under these conditions, and this has to be considered especially in view of the fact that the corpus luteum does not inhibit the growth of the mammary gland. On the other hand we must take into consideration the fact that follicles mature also during the normal cycle and that we do not find so marked a proliferation of the mammary gland during the normal cycle as in hysterectomized guinea pigs. It is therefore very possible that we may attribute this additional growth of the mammary gland to the action of the corpora lutea in both pregnant and hysterectomized guinea pigs. However, in pregnant guinea pigs the development of the mammary gland seems to proceed somewhat further than in hysterectomized animals. Thus in the former we find typical milk secretion towards the end of pregnancy, while in hysterectomized guinea pigs this seems to take place only in exceptional cases.

There is a further similarity in the conditions found in pregnant and in hysterectomized guinea pigs. In pregnant guinea pigs a placenta develops in the uterine mucosa, previously sensitized by the corpus luteum, under the influence of the stimulation of the developing ovum, while in hysterectomized guinea pigs, as well as in normal animals during the ordinary cycle, placentomata may be experimentally produced through mechanical stimulation of various kinds. In both pregnant and hysterectomized animals the proliferation of the decidual cells does not continue during the whole period that the corpus luteum persists, but it ceases after some time, notwithstanding the continued life of the corpus luteum. Furthermore, in both cases the lack of ovulation prevents an early degeneration of the placenta or placentomata. Placental tissue, situated favorably, may persist for a long time in the hysterectomized animals, although great parts of it become necrotic even though the corpus luteum is still active. It is conceivable that contact with the developing embryonal tissue may, in some respects, act favorably on the preservation of the decidual tissue,

insuring for it a better circulation, although on the other hand the embryonal tissue exerts an invading and destructive action on the placenta which is lacking in the case of the placentomata of the hysterectomized animals.

Hysterectomy and also pregnancy in guinea pigs thus produce, in a large measure, their characteristic effects on the sexual system through their action on the corpus luteum. In the former the effects seem to be completely dependent on the persistence of the corpus luteum; in the case of pregnant guinea pigs however there exists, as we have seen, another factor in addition to the corpus luteum, namely, the function of the embryonal tissue, the action of which, in certain respects, needs further analysis.

10. *On the mechanism through which extirpation of the uterus in the guinea pig leads to a marked prolongation in the life and function of the corpus luteum.* The problem, as to the mode through which the hysterectomy exerts its specific effect on the corpus luteum, cannot yet be considered as definitely solved at the present time. Several facts, however, we can hold as established. Thus we have seen that a partial extirpation of the uterus, while it still increases the duration of life and function of the corpus luteum and delays ovulation, has an effect which is much less marked than a more complete extirpation. Even mere incisions into the uterus cause a slight delay in ovulation and probably likewise retard slightly the retrogression of the corpus luteum. We may consider three possible explanations of the effects of hysterectomy:

1. A special nervous mechanism might regulate these processes and we may conceive of the possibility that a coördinating ganglion is extirpated simultaneously with the extirpation of the uterus. However, this interpretation is improbable if we consider the fact that it is unimportant which part of the uterus is extirpated and which part is left in case of incomplete extirpation. It can not therefore be a question of the removal of a certain, definitely localized and coördinating nervous center. 2. There is the possibility that through the extirpation of the uterus we remove an organ that competes with the ovary and especially the corpus luteum in its demand on the blood supply. After elimination of the uterus and thus cutting off the blood supply from the uterus, the corpus luteum is assured of a long continuing, satisfactory circulation. However, the fact that the development of placentomata, which, at least for some time, require a good blood supply and which might thus detract it from the ovary do not interfere with the effect of hysterectomy, renders this interpretation also improbable. 3. It is possible that the uterus, in particular its mucosa, produces an internal secretion which exerts a specific, abbreviating effect on the life of the corpus luteum. The ineffectiveness of transplanted pieces of uterus in changing the results of an almost complete, into those of a partial, extirpation of the uterus seems to be unfavorable to this interpretation. However, we must consider the fact that the amount of

uterine tissue remaining alive and functioning after transplantation is after all relatively small. At present therefore the question as to the mechanism through which extirpation of the uterus exerts its specific effect on the corpus luteum cannot yet be definitely answered.

If we now consider the manner in which pregnancy prolongs the life and function of the corpus luteum, we might attribute it to the development of the embryo, which somehow may improve the blood supply of the corpus luteum. But against this interpretation we may cite our observation, made in a case in which we produced an extrauterine pregnancy experimentally (4). In this case the embryonic tissue, developing in the outer portion of the wall of the uterus, did not prevent the retrogression of the corpus luteum and subsequently a new ovulation. Conversely, we have shown that during pregnancy the uterus is in a somewhat atrophic condition, in which it lacks responsiveness to the hormones of the corpus luteum. We must therefore consider the possibility that during pregnancy the uterus is not functioning normally and that the attachment of a growing embryo to the uterine mucosa makes the uterus ineffective, as far as its action on the corpus luteum is concerned and therefore leads to effects similar to those caused by hysterectomy. However, we do not wish at present to exclude the possibility that, in addition, the normally developing embryo may not also cause a prolongation of life of the corpus luteum.

11. *On the effects of hysterectomy on the generative system in rats.* In a number of white rats we extirpated the uterus without observing a noteworthy delay in ovulation. At the time of examination, which varied between fifteen and forty-five days following the operation, we usually found in the ovaries of these hysterectomized animals various sets of corpora lutea and among them, a number of times, corpora lutea, originating in follicles which had ruptured within the last few days. The vagina underwent the usual proliferative changes into squamous keratinizing epithelium and differed thus markedly from the vagina of the hysterectomized guinea pigs. The mammary glands did not show the marked proliferation which we observed in hysterectomized guinea pigs. We may therefore conclude that contrary to our findings in guinea pigs, hysterectomy in rats has no pronounced effect on the sexual cycle, although at present we cannot deny that it may prolong it to a slight extent; but even such a slight delay has not yet been definitely established by us. The decisive and far reaching effects of hysterectomy in the guinea pig make it very improbable that we have to deal in this species with a peculiarity. It is more probable that the result obtained in guinea pigs indicates a relation between the uterus and corpus luteum which is potentially present in other species of animals, but which in certain cases is affected by secondary conditions. Thus it is conceivable that the corpus luteum differs in the power of resistance to injurious conditions in different species, and that it is presuma-

bly more resistant in those species in which the sexual cycle is longer, and, on the other hand, more sensitive to unfavorable influences in those species in which the sexual cycle is relatively short, as for instance, in rat and mouse. According to this interpretation the sexual cycle in these two kinds of animals would be short because either in the corpus luteum as such, owing to certain peculiarities of its organization, or in the constituent lutein cells, we find a lower power of resistance in these than in other species. The early degeneration of the corpora lutea allows an early ovulation and an early onset of a new cycle. In such animals the effect of hysterectomy, while present in the same way as in the guinea pig, would not be able to overcome the consequences of the great sensitiveness and lability of the corpora lutea. This conception would thus correlate in a causal way the differences in duration of the sexual cycle and the differences in the effects of hysterectomy in different species, with variations in the power of resistance of the corpora lutea or its constituent cells to injurious factors. We offer this theory as a purely tentative and provisional explanation and we consider it possible that other factors, either alone or in combination with the factor we have suggested, are responsible for the differences found in different species of animals.

SUMMARY

1. A complete or almost complete hysterectomy in the guinea pig prolongs very considerably the life and function of the corpus luteum. Thus the corpus luteum is able under these conditions to exert typical effects on the periodicity of the sexual cycle, and on the system of sex organs, especially also on the mammary gland; and, as we stated in a previous communication, it has the power of counteracting the effect of follicular extract on the vagina.

2. It can be shown experimentally that hysterectomy does not as such lead to a suspension of ovulation or of the proliferation of the vaginal epithelium, but that it is the persistence of the corpus luteum which exerts these effects and that hysterectomy acts directly on the corpus luteum and only indirectly on the other processes.

3. Through hysterectomy the essential changes characteristic of pregnancy can be reproduced in the system of sex organs of the guinea pig. Some differences exist, however, between these two conditions, as far as the reaction of the uterus to the corpus luteum hormone and the intensity or frequency of milk production in the mammary gland are concerned. This similarity between the effects of pregnancy and of hysterectomy on the life and function of the corpus luteum suggest the possibility that also in pregnancy a functional inactivation of the uterine mucosa is responsible, at least partially, for the effects of this state on the prolongation of life and

function of the corpus luteum and indirectly also for the consequences resulting from the activity of the latter organ.

4. A partial hysterectomy leads to similar but quantitatively much less marked changes than a complete or almost complete hysterectomy; even incisions into the uterine horns somewhat prolong the sexual cycle. These effects of a partial extirpation of the uterus concern especially the first sexual period following the operation, and they decrease or are lost in the following periods.

5. Transplantation of pieces of uterus cannot change the effects of a complete, into those of an incomplete, hysterectomy.

6. We have not found it possible so far to prolong through hysterectomy the growth period of experimental placentomata to the same extent that we could prolong the life and function of the corpus luteum; while the absence of ovulations during the period of survival and function of the corpus luteum protects the placentomata against the injurious effects of ovulation and thus indirectly may contribute to the prolongation of life of the placentomata, they usually begin to become necrotic at a time when the corpora lutea are still functioning.

7. Tentatively we suggest that differences in the effects of hysterectomy on the system of sex organs and on the periodicity of the sexual cycle in different species may at least partially depend upon differences in the power of resistance to unfavorable conditions on the part of the corpus luteum as such, or of the lutein cells in these species.

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THE SIGNIFICANCE OF THE PERICARDIUM IN ACUTE CARDIAC DILATATION PRODUCED BY ANOXEMIA

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It has been known for a long time that the pericardium may in certain conditions affect the action of the heart and also may affect the circulation. The influence of an increase in intrapericardial pressure was first suggested by Morgagni as early as 1761. Cohnheim (1889), Francois-Franck (1897), Lewis (1908) and others proved the physiological significance of a rise in intrapericardial pressure. They found that an intrapericardial pressure which is a little above the venous pressure may interfere with the diastolic filling of the heart.

Barnard (1898) took strips of the fibrous layer of the pericardium and subjected them to traction. He concluded that the pericardium is inextensible. He also subjected the heart with its pericardium removed to pressure and found that it ruptured at $\frac{3}{4}$ to 1 atmosphere, whereas with the pericardium intact it ruptured at $1\frac{1}{4}$ to $1\frac{3}{4}$ atmospheres. His conclusions from these observations were that the pericardium is important in normal life for the prevention of acute cardiac dilatation.

Kuno (1917), working with the heart-lung preparation, concluded that the pericardium is necessary for the unimpaired working of the heart in normal life.

Felix (1925) induced valvular and other lesions in dogs' hearts and attempted by modifying the pericardium to influence these valvular defects. He found that making the pericardium smaller hampers heart action while making it larger had favorable influence on certain induced lesions, particularly mitral defects.

The latest work is that of Wilson and Meek (1927). They made the interesting observation that in the dog the apparent restraining action of the pericardium on the diastole at low venous pressure is due to the tonus of the diaphragm, which affects the heart through the pericardio-diaphragmatic attachments. With these attachments severed they found that at an effective venous pressure of about 15 cm. of water the dog's heart in diastole completely fills the pericardium.

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Rehn (1913) studied 5 dogs in which the pericardium had been partially or totally removed and reported no ill effects on the health of the dog. Mazzone (1912) removed the pericardium in 7 dogs and came to the same conclusion. Yamada (1917) slit the pericardium in a series of 14 dogs. He then subjected these animals to exercise during the post-operative period. He found no evidence of dilatation or other important pathological changes. Yamada concluded from these experiments that the protective influence of the pericardium was not very important. He differed, therefore, from the views of Barnard and Kuno. Beck and Moore (1925) removed the pericardium in 5 animals and subjected them to severe exercise. These authors found that the animals responded to severe exercise very well and in no way did the general health seem to be impaired. A number of clinical cases have been reported of partial or total congenital absence of the pericardium (Moore, 1925; Grant, 1926). These conditions did not appear to produce any clinical symptoms and the death of these patients seemed in no way caused or hastened by these congenital defects.

Since there is still a divergence of opinion in regard to the importance of the pericardium, it was thought worth while to study the influence of the pericardium on the acute dilatation of the heart induced by anoxemia, endeavoring to maintain the animals in as normal physiologic condition as possible.

PROCEDURE. Barbitalized dogs, cats and monkeys were used. In the case of dogs and cats barbital was given intravenously; in the monkeys intramuscularly. An x-ray picture was now taken for the control. The distance from the plate to the center of the tube was one meter. The length of exposure varied from 2 to 3 seconds. Immediately after the control picture was taken, the animal was placed in a steel respiratory chamber (Kolls and Loevenhart, 1915) and subjected to anoxemia. The same apparatus and methods were used as described by the senior author in a previous paper (Van Liere, 1927). Eight minutes were allowed to bring the pressure down to the desired level and it was held there for 2 minutes. The animal was removed and the x-ray picture taken again; removing the animal and taking the x-ray picture required approximately 25 seconds. It was aimed to place the animal in the identical position as the control.

A tracheal cannula was now inserted and artificial respiration was administered. A small opening was made in the 4th or 5th interspace in the approximate region of the cardiac apex. The pericardium was grasped with a small hemostat and was slit longitudinally its entire length or it was entirely removed—generally the latter was done. The wound was partially closed, the lungs were then expanded and held thus for a few seconds until the remainder of the wound was closed. The artificial respira-

tion was now discontinued and the animal allowed to resume its normal breathing.

The animals were allowed two hours to recover from the effects of the operation. A control x-ray picture was again taken. The animal was placed in the respiratory chamber and subjected to anoxemia. It was aimed to duplicate as closely as possible the procedure previous to the removal of the pericardium as described above.

In order to check the results obtained with the use of the respiratory chamber, the nitrous-oxide oxygen apparatus was used as described in a previous paper (Van Liere, 1927). The results compared favorably to those obtained with the respiratory chamber.

It may be stated here that considerable difficulty was experienced in taking x-ray pictures of the heart after removal of the pericardium. After the heart has lost its support of the pericardium it is very apt to rotate, especially toward the left. It was found necessary to place the animal exactly in an antero-posterior position so that the heart would lie in its correct position; the fluoroscope was found of value here. In several cases a number of control x-ray pictures were taken to check our results. In the table, however, only the average figure is given.

The cardiac silhoutte was traced with a pen and the area measured by means of the planimeter.

RESULTS. The following table gives the results obtained:

TABLE 1

ANIMAL	WEIGHT	CARDIAC AREA		INCREASE IN CARDIAC AREA IN ANOXEMIA	ANOXEMIA OXYGEN	CARDIAC AREA, PERICARDIUM CUT		INCREASE IN CARDIAC AREA IN ANOXEMIA	ANOXEMIA OXYGEN
		Normal	Anoxemia			Normal	Anoxemia		
	kgm.	*sq. cm.*	*sq. cm.*	*per cent*	*per cent*	*sq. cm.*	*sq. cm.*	*per cent*	*per cent*
Dog 1.....	7.8	32.89	38.05	15.68	3.0	32.25	36.95	14.60	3.0
Dog 2.....	17.	56.43	65.14	15.43	3.50	63.85	70.62	10.61	4.0
Dog 3.....	3.1	19.35	20.96	8.33	4.50	22.38	24.63	10.08	4.50
Dog 4.....	5.1	27.09	29.99	10.71	4.45	27.41	29.34	7.06	5.40
Dog 5.....	4.5	24.18				27.60	29.67	7.50	5.0
Dog 6.....	6.3	32.50				33.41	35.60	6.55	5.0
Cat 1.....	2.	10.32				10.50	12.12	15.32	4.0
Cat 2.....	2.5	13.41				13.54	15.15	11.90	4.0
Cat 3.....	2.3	12.38				14.83	16.25	9.57	5.0
Monkey 1...	2.3	10.64	12.64	18.79	3.0	10.64	13.22	24.24	3.0
Monkey 2...	2.8	11.73	15.15	29.04	3.0	11.93	16.77	40.57	3.0

TABLE 2

DATE	CARDIAC AREA				INCREASE IN CARDIAC AREA			COMMENTS
	Normal	Anoxemia						
		5 per cent O_2	4 per cent O_2	3 per cent O_2	5 per cent O_2	4 per cent O_2	3 per cent O_2	
Monkey 1; weight 2.3 kgm.								
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*per cent*	*per cent*	*per cent*	
May 2....	11.15	12.06	12.64	12.64	8.06	13.26	13.26	No anesthetic
May 4....	11.03	11.93	12.32	12.32	8.20	11.70	11.70	No anesthetic
May 5....	11.09	12.58	12.77	12.77	13.44	15.15	15.15	No anesthetic
May 10....	11.09	12.19	12.64	12.64	9.92	13.97	13.97	No anesthetic
May 12....	10.64	12.25	12.25	12.64	15.13	15.13	18.79	Barbital anesthesia
May 12....	10.64	12.25	12.58	13.22	15.13	18.23	24.24	Pericardium cut
Monkey 2; weight 2.8 kgm.								
May 9....	13.87	15.09	16.90	17.22	8.80	21.85	24.15	No anesthetic
May 10....	14.19	16.45	17.42	18.06	15.92	22.76	27.27	No anesthetic
May 14....	13.55	16.13	16.77	17.09	19.04	23.76	26.12	No anesthetic
May 25....	11.74	12.58	13.22	15.15	7.15	12.60	29.04	Barbital anesthesia
May 25....	11.93	13.22	13.55	16.77	10.81	13.57	40.56	Pericardium cut

Discussion. It appears from the data obtained from the cats and dogs (table 1) that the heart with the pericardium removed seems to withstand anoxemia about as well as the heart with the pericardium intact. This is, however, more apparent than real. On the whole less severe degrees of anoxemia were used in the cases where the pericardium had been removed. It must also be pointed out that unless approximately 2 hours were allowed to elapse after the operation for the removal of the pericardium, the heart would not be back to its normal size. In some of these animals, particularly in the early work, where the importance of this factor was not fully recognized, a sufficient time was not allowed for the heart to return to its normal size. This also then in part explains the rather small increase in cardiac area in some of the animals with the pericardium removed. In some of the animals, moreover, 2 hours was not sufficient time for the heart to return to its normal size, but as these were acute experiments with the animals under barbital it was thought advisable not to wait over 2 hours, as if a longer time was allowed to elapse the physiologic condition of the animal might be impaired. Obviously the proper way to check this is to allow complete recovery; this work is contemplated in the near future.

In the animals, furthermore, with the pericardium removed if the anoxemia was produced somewhat too rapidly or if the grade of anoxemia was a little severe, respiratory failure occurred earlier than in the normal ani-

mals, and in some of these animals an anoxemia of 7 to 10 per cent oxygen produced a sudden and acute cardiac dilatation from which the heart did not recover. This was never seen with the pericardium intact. In the former cases the cardiac silhouette showed an enormous increase (35 to 40 per cent) above the normal. These data were not included in the table. There is thus considerable individual variation.

Table 2 shows the results obtained with monkeys (Macacus rhesus). It will be noted that controls were run on different days. On the whole it will be seen that the normal cardiac area obtained on different days check very closely. The reason for this is that several control x-ray pictures were taken at the time of each experiment and the average cardiac area was used. In many instances, furthermore, several x-ray pictures were taken of the heart under a definite per cent of anoxemia; again, however, only the average figure is given in the table. It will be further noticed that the heart in the barbitalized monkeys showed a smaller area than the unanesthetized animals. A possible explanation for this is that these latter animals were as a rule frightened and restless when they were subjected to the experiment. The results obtained with monkeys seem to show more clearly than in the other animals that the pericardium is perhaps only important in severe stress. In ordinary circumstances it probably is not very important in regard to the prevention of cardiac dilatation, but in conditions which throw a great stress on the heart it probably plays a protective rôle in that it prevents the cardiac muscle from undue stretching, which might permanently injure the muscle fibers.

The table below shows the effects of extreme anoxemia on the heart:

Pericardium intact		*Pericardium cut*	
Animal 1	18.64%	Animal 1	29.68%
Animal 2	20.67%	Animal 2	30.76%
Animal 3	25.73%	Animal 3	36.0%
		Animal 4	40.0% (cat)

The animals of the above table were subjected to anoxemia in the respiratory chamber and kept there until respiratory failure occurred. They were then quickly removed and the x-ray picture taken. The heart was still beating when the animals were removed and in most instances artificial respiration would restore them to normal breathing. The animals used were dogs except animal no. 4, which was a cat.

CONCLUSIONS

1. Animals with the pericardium removed tend to show a greater cardiac dilatation in severe degrees of anoxemia, especially in monkeys.

2. The protective action of the pericardium as regards the prevention of acute cardiac dilatation probably never comes into play in the normal animal, except possibly in instances of extreme stress on the heart.

We take great pleasure in thanking Professor Carlson for his constructive criticism throughout this work.

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THE RÔLE OF TOXINS IN PARATHYROID TETANY

I. An Attempt to Control Tetany by the Oral Administration of Kaolin

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In previous studies by the senior author in coöperation with Doctor Fisher (Fisher and Larson, 1925), (Larson and Fisher, 1927 a, b), it has been demonstrated that the maintenance of a normal level of the calcium of the blood is essential if tetany is to be controlled properly. We are well aware of the fact that after the control of the acute attacks of tetany for periods of ten to thirty days, tetany may not supervene even if the level of the blood calcium is below normal. But a rapid lowering of the calcium is responsible for tetany. This has been previously demonstrated by Salvesen (1923 a, b). The calcium theory of tetany is well supported by the work of MacCallum (1911), Collip (1925) and many others.[2]

Consequently when Dragstedt and Sudan (1926) reported their results on the prevention and control of parathyroid tetany by the oral administration of kaolin it was deemed advisable to repeat their experiments in order to study the mechanism of the action of kaolin. We were first interested in determining whether a normal blood calcium was maintained after the administration of kaolin to a thyro-parathyroidectomized dog. We were also desirous of determining whether guanidine which has been thought by many investigators, notably Koch (1912), Paton and Findlay et al. (1917) and Dragstedt et al. (1923 a, b) to be the causative factor in the production of tetania parathyreopriva would be absorbed by kaolin.

Experimental procedure. A series of twenty dogs were thyro-parathyroidectomized by removing the entire thyroid capsule with the contained thyroid and parathyroid glands. The blood vessels were ligated at least one inch from the glands so as to be reasonably certain that the parathyroids were removed. The diet consisted of kitchen scraps which were composed largely of carbohydrates. Twelve of the dogs were given

[1] An abstract of the thesis submitted by Leo A. Elkourie in partial fulfilment of the requirements for the degree of Master of Science.

[2] For a review consult J. B. Collip, Medicine, 1926, v, 1.

kaolin and mineral oil by stomach tube daily. The other eight dogs were operated in the same manner and cared for exactly the same way except that no kaolin was administered. The tetany was usually controlled by intravenous injections of calcium lactate.

The percentage of calcium in the blood serum was determined by the Clark-Collip method (1925). The guanidine analyses were made by the method of Pfiffner and Myers (1926). In determining the calcium content of the kaolin (Merck's) 10 gram samples were suspended in 100 cc. of 0.4 per cent HCl and kept at 37.5°C. for one hour. After removing the kaolin by filtration the calcium was determined by a standard volumetric method (Smith, 1921).

In-vitro tests were made to determine whether kaolin would absorb guanidine in either acid or alkaline medium. The tests were made as follows: 10 cc. of guanidine solution (1 cc. = 0.5 mgm.) was added to 10 cc. of 0.4 per cent HCl and 1 gram of kaolin (Merck). As a control 10 cc. of the same guanidine solution were added to an equal amount of 0.4 per cent HCl. The solutions were then kept at 37°C. for the required time (1 to 4 hours). In order to keep the kaolin suspended the flasks were shaken thoroughly every five minutes. At the end of the period the kaolin was removed by filtration and a guanidine analysis made by the method of Pfiffner and Myers (1926). Two similar series in which 0.05 per cent sodium carbonate and 0.25 per cent sodium bicarbonate mediums replaced the hydrochloric acid were handled in the same way.

An in-vivo experiment to determine the absorption of guanidine from the intestine of an anesthetized animal was also performed. Under ether anesthesia, an incision in the anterior abdominal wall of a dog was made. After isolating a loop of the ileum and a loop of the colon about a gram of guanidine in solution in water was placed in each of the loops. These solutions were allowed to remain in the loops for a period of one hour. After removal from the loop, the solutions were treated by the Folin-Wu procedure to remove any protein material and the guanidine content determined as before.

Dog K-1. Female. Weight, 9 kilos.

12– 8–26 10:00 a.m. Thyro-parathyroidectomized.
12– 9–26 3:00 p.m. Impending tetany; 10 grams calcium acetate, 50 grams kaolin and 50 cc. of oil by stomach tube.
12–10–26 11:00 a.m. Impending tetany; 10 grams calcium acetate.
12–11–26 10:00 a.m. 50 grams kaolin and 50 cc. oil.
11:00 p.m. Tetany, 50 grams kaolin and 50 cc. oil.
12–12–26 9:30 a.m. Indications of tetany, 50 grams kaolin and 50 cc. oil.
12:30 p.m. Decided tetany so 6 grams of calcium lactate were injected subcutaneously. The calcium of the blood serum was 6.28 mgm. per 100 cc. at this time.
12–13–26 a.m. 50 grams of kaolin and 50 cc. oil.
p.m. Same as a.m.

12-14-26 9:30 a.m. Impending tetany, 50 grams kaolin and 75 cc. oil. Blood calcium 6.03. Later the tetany became more severe so 8 grams of calcium lactate were injected.
12-15-26 50 grams kaolin and 100 cc. oil.
12-16-26 50 grams kaolin and 100 cc. oil.
12-17-26 50 grams kaolin and 100 cc. oil.
12-18-26 Sacrificed. Blood serum calcium 6.55 mgm. per 100 cc.

Dog K-20. Female. Weight, 11 kilos. This dog was given 100 grams of kaolin and 100 cc. of mineral oil daily.
4- 5-27 2:30 p.m. Thyro-parathyroidectomized.
4- 6-27 10:30 p.m. No tetany but 50 mgm. of calcium given as a preventive measure.
4- 7-27 Same as 4-6-27.
4- 8-27 5:30 p.m. Tetany; 100 mgm. of calcium.
4- 9-27 4:00 p.m. Tetany; 100 mgm. of calcium.
11:00 p.m. Tetany; 100 mgm. of calcium.
4-10-27 3:00 p.m. Tetany; 100 mgm. of calcium.
11:00 p.m. Tetany; 100 mgm. of calcium.
4-11-27 8:45 a.m. Tetany; 100 mgm. calcium.
10:30 p.m. Preventive dose—50 mgm. calcium.
4-12-27 11:00 a.m. Tetany; 100 mgm. calcium.
4-13-27 4:30 p.m. Tetany; 100 mgm. calcium.
4-14-27 8:30 a.m. Tetany; 100 mgm. calcium.
4:15 p.m. Tetany; 100 mgm. calcium.
4-15-27 9:00 a.m. Tetany; 100 mgm. calcium.
7:15 p.m. Tetany; 100 mgm. calcium.
4-16-27 10:00 a.m. Tetany; 100 mgm. calcium.
4-17-27 2:00 p.m. Tetany; 100 mgm. calcium.
4-18-27 8:00 a.m. Dead. Tetany.

Dog K-15. Female. Weight, 9 kilos. Control animal. No kaolin administered.
3-17-27 2:15 p.m. Thyro-parathyroidectomized.
3-18 and 3-19 No treatment.
3-20-27 Impending tetany; 75 mgm. calcium.
3-21-27 No treatment.
3-22-27 9:45 a.m. Tetany; 100 mgm. calcium.
3-23-27 3:15 p.m. Tetany; 100 mgm. calcium.
3-24-27 11:00 a.m. Tetany; 75 mgm. calcium.
3-25 to 3-28 No treatment, refused food.
3-29-27. Dead. Tetany.

Dog. K-16. Male. Weight, 7 kilos. Control animal. No kaolin administered.
3-17-27 2:00 p.m. Thyro-parathyroidectomy.
3-18-27 No treatment.
3-19-27 9:15 a.m. Tetany; 75 mgm. calcium intravenously.
3-20-27 4:30 p.m. Impending tetany; 75 mgm. calcium intravenously.
3-21-27 No treatment.
3-22-27 Normal.
3-23-27 10:45 a.m. Tetany; 75 mgm. calcium intravenously.
3-24-27 No treatment.

3–25–27 9:00 a.m. Tetany; 75 mgm. calcium intravenously.
3–26–27 3:00 p.m. Dead. Autopsy revealed no accessory parathyroid tissues.

Guanidine absorption by kaolin at 37.5°C.

INCUBATION PERIODS	MEDIUM	GUANIDINE USED	RECOVERED	RECOVERED FROM CONTROLS
hours		*mgm.*	*mgm.*	*mgm.*
1	0.2 per cent HCl	5	5.12	5
4	0.2 per cent HCl	5	5.06	5
2	0.05 per cent Na_2CO_3	5	5.09	4.95
2	0.25 per cent $NaHCO_3$	5	5.02	4.95

Non-survival experiment on dog to test guanidine absorption in colon and ileum

PERIOD IN LOOP	PORTION OF ALIMENTARY CANAL USED	AMOUNT OF GUANIDINE	AMOUNT OF GUANIDINE RECOVERED
hours		*mgm.*	*mgm.*
1	colon	995.6	1011.7
1	ileum	1035.7	1030.6

Discussion. Though this investigation was originally started with the idea in mind of determining the reason for the alleviation of tetany by kaolin as claimed by Dragstedt and Sudan (1926), it became evident that at least in our hands kaolin was relatively ineffective. In our observations on twelve dogs to which kaolin was administered daily it seemed as if kaolin had no effect whatsoever except possibly in one specific attack of tetany (dog K-1 on 12–11–26). To make sure that we were not drawing any unwarranted deductions when we concluded that kaolin did not prevent nor modify the tetanic attacks, we operated eight dogs which were treated in exactly the same way as the animals which received kaolin. The protocols which are included in this article are typical of the animals which we observed and to our minds there is no difference between those which received kaolin and those that did not receive kaolin. An analysis of the protocols of the thyro-parathyroidectomized dogs reported by Dragstedt and Sudan (1926) indicates that some of their assumptions are not warranted. In every one of the ten dogs whose protocols are included in this report of Dragstedt and Sudan (1926) with the exception of dog 85 which died during the third day in the first attack of tetany, accessory treatment mainly in the form of Ringer's solution or calcium lactate had to be resorted to in order to prolong the lives of the animals. For instance, dog 50 required calcium frequently in the form of either Ringer's solution, calcium lactate or milk in order to control the tetany. In the case of dog 50, eight doses of calcium lactate besides Ringer's once and milk at least four times were necessary. With dog 52 which lived 15 days Ringer's solution had to be injected five times in order to relieve the tetany even if 200 grams of

kaolin were administered the first two days and 100 grams during the remainder of the period. Both calcium lactate (Luckhardt and Goldberg, 1923) and Ringer's solution (Luckhardt and Rosenbloom, 1921) have been shown to be effective in the relief of tetania parathyreopriva. It seems to us that any method which is at all efficacious should not require that much accessory treatment. The rapidity of the onset, severity, and recurrence of the attacks seem to be the same among the animals which received kaolin as that of the control animals. We were unable to distinguish any difference in any respect.

A normal blood calcium was not maintained in these animals by kaolin treatment. The blood analysis of the animals always showed a low blood calcium as is evident from the protocols. And any beneficial results ascribed to kaolin cannot be due to its calcium content, since a careful chemical analysis showed only 0.01 per cent which would give only 10 mgm. of calcium per 100 grams, an insignificant amount.

Guanidine which has been claimed by many investigators, notably Koch (1912), Paton, Findlay, et al. (1917) and Dragstedt, et al. (1923 a, b) to be the causative factor in parathyroid tetany was not absorbed by kaolin. We tried this experiment in both an alkaline and an acid medium being careful to keep the kaolin well suspended. Our results showed that there was no absorption in any case. Though Dragstedt may be correct in his assumption that kaolin does absorb toxins it does not absorb the hypothetical toxin, guanidine, from either an acid or alkaline medium. The action of kaolin in the gastro-intestinal tract in the rat when it comprises 2 per cent of the total diet, according to Bergeim (1924–1925) is to cause a decrease and then an increase in intestinal reduction. Since intestinal reduction is a good index of intestinal putrefaction this should be an unfavorable condition if Dragstedt's assumptions are correct.

That the intestine of an anesthetized animal does not absorb guanidine readily does not seem remarkable because Swingle and Nicholas (1924) have shown that even 2 grams of guanidine salts per kilo of body weight have not caused either tremors or convulsions. From our results it would seem that the relatively low toxicity of guanidine and its salts is due to the fact that they are not easily absorbed from either the ileum or the colon. That there was no absorption from the colon is significant in view of the results of Oldberg and Ivy (1926). These investigators found that colectomy delayed and moderated the symptoms of tetany following thyroparathyroidectomy. It would seem logical to assume that this amelioration was due to the elimination of the absorption of toxins but it can be more readily explained on a calcium basis. The colon is one of the pathways for the elimination of calcium (Bergeim, 1926). It follows of course that calcium would not be as readily eliminated after colectomy.

CONCLUSIONS

1. Kaolin orally does not have any decided effect in preventing or controlling tetania parathyreopriva.

2. Kaolin does not absorb guanidine in vitro from either an acid or alkaline medium.

3. Guanidine is not absorbed in significant amounts from either the ileum or the colon of an anesthetized dog during a period of one hour.

We wish to express our thanks to Dr. Robert M. Oslund for revising the manuscript.

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STUDIES ON THE ANEMIA OF RICE DISEASE

The Effects Produced by the Addition of Betaine Hydrochloride, Lactose, Vitamins A and C, Magnesium Sulphate or Mineral Oil to the Polished Rice Diet of Pigeons

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Numerous hypotheses have been advanced tending to explain the cause of the development of the phenomena of rice disease; among the most important are the original toxin theory of Eijkman (1897) which in recent years has been altered to include the condition of auto-intoxication (Walshe, 1918, 1920), a deficient gastro-intestinal secretory activity (Lumiere, 1920), and a subnormal state of oxidative activity (Abderhalden, 1922); Anderson and Kulp (1922) have stated that vitamin B lack is the predisposing factor in beriberi and that the physiological changes which subsequently develop (McCarrison, 1921–1922) may be associated with the degree of inanition which is invariably present. Most of the existing observations could be interpreted by means of any of these hypothesis and none are at present disproven. It is quite conceivable that several or all may play a part and that some at least would be of secondary consequence, although the order of the causal chain has not been determined.

In a previous correlation of the body weight, respiration and temperature changes coincident with the development of rice disease in pigeons (Barlow, 1926), a marked increase in the number of bacteria in the blood stream was observed as previously noted by McCarrison (1923) and Cramer (1923), and the degree of the bacterial invasion seemed inversely proportioned to the decrease in the red corpuscle count and hemoglobin level respectively.

On the working theory that this bacteremia which developed in the course of the rice disease was the consequence of a gastro-intestinal stasis, it appeared worth while to determine whether it could be influenced by one or more of several procedures: first, increasing the acidity of the digestive juices, since the latter are subnormal (Lumiere, 1920; Anderson and Kulp, 1922; Michel and Koskowski, 1922); second,

through the addition of vitamins A and C, in order to correct the rice diet in so far as a deficiency of these vitamins was concerned; third, to alter the character of the intestinal flora by increasing the acidophilic bacteria through the administration of lactose; fourth, to alter the degree of absorption from the gastro-intestinal tract and to modify the tendency to stasis through the administration of magnesium sulphate or mineral oil respectively.

METHODS. Adult pigeons averaging 360 grams in weight were used. All birds were placed on a mixed whole grain diet and grit until a constant level of body weight had been established, after which the control observations including body weight, respiration rate, rectal temperature, red corpuscle count, hemoglobin and blood sugar levels were correlated at seven day intervals over a three week period. The observations were then continued at similar intervals throughout the period of study. The blood samples necessary for making the red corpuscle counts, hemoglobin and blood sugar determinations were obtained by a single puncture of a small wing vein without the use of an anesthetic. The hemoglobin was estimated by the Tallquist scale and the blood sugar level by the picric acid method of Epstein, using 0.1 to 0.2 cc. of blood.

The pigeons were then divided into several groups and their diets altered during the succeeding twenty-eight days as follows: group A (36 birds),

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Figs. 1 and 2 represent the median data for each of the observations noted of all birds of each respective group, as follows:

Group A—Normal diet
Group B—Polished rice diet
Group B (W)—Polished rice diet—data from Weill et al. (1922)
Group C—Fast plus water
Group D—Polished rice plus 1 gram lactose per bird per day
Group E—Polished rice plus betaine HCl 0.3 to 0.6 gram per day
Group F—Polished rice plus 2 grams butter plus 5 cc. orange juice per day
Group G—Normal diet plus 0.4 gram magnesium sulphate per bird per day
Group H—Polished rice diet plus 0.4 gram magnesium sulphate per bird per day
Group I—Normal diet plus 5 cc. mineral oil per day per bird
Group J—Polished rice diet plus 5 cc. mineral oil per day per bird.

Fig. 3 represents the median net wet and dry daily excretion level per bird of a series of pigeons on a normal diet before, during and after the administration of magnesium sulphate or lactose. The shaded blocks on the abscissa represent the periods during which the cathartics were administered. The ordinate gives the percental change of the daily excretion level from the normal.

Fig. 4 represents the total wet excretion level per pigeon of birds on a polished rice diet (solid line), polished rice plus magnesium sulphate (dash line), and polished rice plus lactose (broken line). The ordinate expresses the percental change of the daily excretion level from that established while on a normal diet. The shaded block on the abscissa represents the period of polished rice feeding; and the time during which the cathartics were administered.

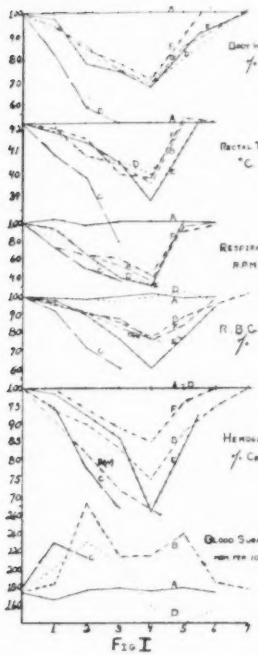

FIG I

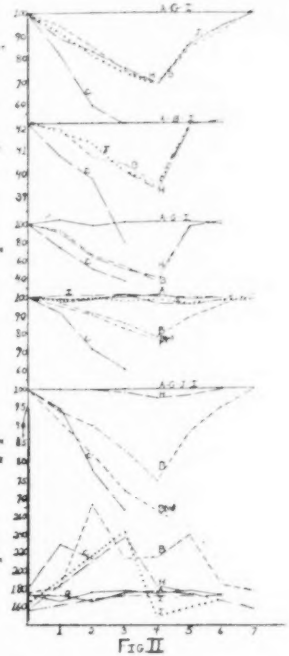

FIG II

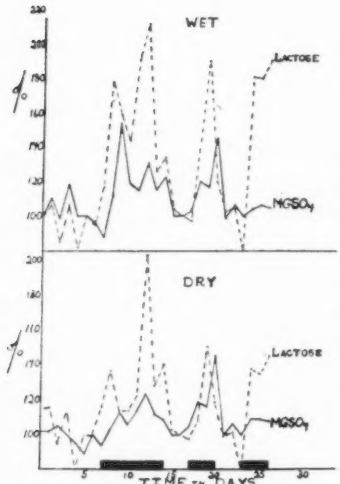

FIG. 3

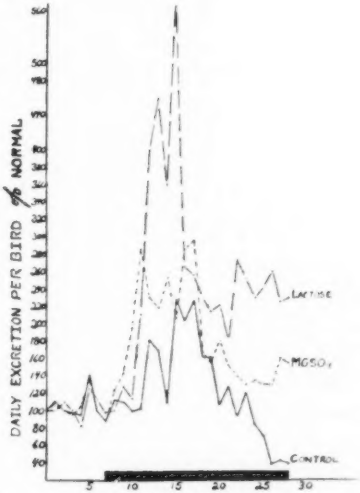

FIG. 4

mixed whole grain; group B (32 birds), polished rice; group C (29 birds), complete fast with access to water; group D (32 birds), polished rice plus 1 gram of lactose per day; group E (12 birds), polished rice plus 0.3 gram betaine HCl per day during the first two weeks and 0.6 gram per day during the third and fourth weeks; group F (12 birds), polished rice plus 2 grams of butter and 5 cc. of orange juice per day; group G (36 birds), mixed whole grain plus 0.4 gram $MgSO_4$ per day; group H (35) birds), polished rice plus 0.4 gram $MgSO_4$ per day; group I (24 birds), mixed whole grain plus 5 cc. of Squibb heavy mineral oil per day; group J (20 birds), polished rice plus 5 cc. mineral oil per day. All observations taken during the experimental feeding period were made 22 to 26 hours after the administration of the last dosage of lactose, magnesium sulphate or mineral oil. At the end of the twenty-eighth day period of study the diets of all surviving birds were changed to mixed whole grain and the observations continued until recovery was complete.

Results. The results as shown in figures 1 and 2 represent the median values for each observation taken for all birds of each respective group as designated by letter. The median control observations taken for each group are represented on the ordinates by a single point and the changes with time during the experimental treatment on the abscissae.

Body weight. From the three control series, i.e., A, B and C, figure 1, one may observe that the weight loss of those birds on a polished rice diet of 28 days' duration corresponds very closely to the half period of the weight loss of pigeons on a complete fast.

The addition of betaine HCl, lactose, vitamins A and C, magnesium sulphate or mineral oil to the polished rice diet had no apparent influence on the usual weight changes to be observed in pigeons on a polished rice diet alone, group B.

The observation of Michel and Koskowski (1922) that the secretion of acid and pepsin diminishes simultaneously and that both may be secreted under proper conditions even during the paralytic stage of rice disease possibly explains the ineffectiveness of betaine HCl to affect the nutrition disturbance of rice disease, since at no time is a condition resembling achlorhydria present. According to Plimmer, 1907, fowls are unable to utilize lactose. Consequently the addition of lactose to the rice diet could only influence the weight changes indirectly, i.e., through the production of a saline cathartic action, similar to the magnesium sulphate action.

Temperature. The rectal temperature changes of all pigeons receiving rice were quite parallel to the loss of body weight, irrespective of any of the procedures used.

Respiration. The respiratory rates of all pigeons receiving rice were quite parallel irrespective of any treatment attempted. The close

correspondence between the respiratory changes of rice disease and complete fasting suggests a close parallelism in the two conditions.

Red corpuscle count. The red cell counts of pigeons on a polished rice diet fall approximately 25 per cent in a four week feeding period. The anemia becomes evident after the second week and is then *progressive*, and like the changes observed for weight and temperature the maximum drop noted is approximately one-half that usually observed in fasting.

The administration of betaine hydrochloride or butter and orange juice in addition to the rice diet had no definite influence on the direction or degree of change to be noted with polished rice alone.

The addition of lactose, magnesium sulphate or mineral oil (J) to the polished rice diet prevented the anemia in a very high percentage of the birds. This beneficial effect might conceivably have been due to a concentration of the blood volume, although such an effect would not be expected, since Underhill and Errico (1922) have shown that the effects of magnesium sulphate on the blood volume of mammals disappear in $2\frac{1}{2}$ to 3 hours following its administration. Confirmation of this point is being extended to pigeons.

Hemoglobin. In general, the changes in the hemoglobin percentages parallel the red count, although the hemoglobin seems to have been depressed less than the corpuscles in the rice plus butter and orange juice series, but probably the variation noted is within the range of statistic error.

Blood sugar. The blood sugar levels were determined during the development of rice disease, in order to check and extend the observations of Funk and von Shonborn (1914) and Tasawa (1915); likewise to determine whether a possible concentration of the blood volume by magnesium sulphate, mineral oil or lactose was the explanation of the absence of the usual rice anemia in pigeons receiving a daily dosage of magnesium sulphate, mineral oil or lactose in addition to polished rice.

A very definite hyperglycemia was observed as a consequence of feeding polished rice, as previously shown by Funk and Shonborn, Tasawa (1915), Hirai (1921), Hosokawa (1924) and Hoski and Ukai (1926). No evidence of blood concentration on addition of magnesium sulphate, mineral oil or lactose to the usual rice diet was obtained through a study of the blood sugar concentration, i.e., the blood sugar levels of pigeons on a normal or polished rice diet, 22 to 26 hours after the administration of magnesium sulphate, lactose or mineral oil, did not differ noticeably from their respective controls.

Excreta. Since the beneficial influence of the several cathartics on the rice anemia was apparently not due to blood concentration, it seemed quite possible that the explanation might be due to a secondary effect on the gastro-intestinal tract, i.e., modification of a tendency to stasis and bacte-

remia through a direct cathartic action. The influence of magnesium sulphate and lactose on the median net weight of excreta per bird of a series of pigeons on a normal and polished rice diet respectively is shown in figures 3 and 4.

By comparing figures 3 and 4, one may observe that only quantitative differences are shown in the net excretion levels of pigeons on either normal or polished rice diets, when magnesium sulphate or lactose was added to the daily diet, i.e., in both groups of pigeons a definite catharsis resulted.

Discussion. The great similarity between reactions to a polished rice diet and complete fasting in the direction of the curves for weight, respiration, temperature, red blood count as well as hemoglobin levels seems to indicate a close relationship between the two conditions, i.e., the polished rice diet apparently results in changes closely allied to pure inanition (Gulick, 1924) and might be described as an incomplete fast, since the degree of change corresponds very closely to the half change in fasting, and since the fatal termination of beriberi is roughly twice as long in period of time, as that in complete fasting.

The administration of loosely bound hydrochloric acid is not beneficial in any way to pigeons on a polished rice diet, in spite of the fact that the acid and pepsinogen production in beriberi is quite low (Michel and Koskovski 1922; Groebbels and Sperfield, 1925).

The administration of vitamins A and C in relatively large quantities had no influence on the usual physiological changes to be observed in beriberi. The weight and respiration changes were negligibly smaller than in the control series on polished rice only, but the differences, if considered definite, might be attributed to the very small vitamin B content of the orange juice (Daniels and Laughlin, 1920) or possibly to the utilization of a fraction of the fat which was administered. The failure of the pigeons, on a polished rice diet, to show a response on the addition of vitamins A and C to their diet, is in contrast to the observation on rats by Koessler et al. (1926) and to those of Suski (1923) on pigeons. However, the data presented confirm the observations of Lax (1921), that beriberi pigeons were unaffected by either cod liver oil or alcoholic extracts of the oil; and of Sugiura and Benedict (1923), who state that pigeons do not require vitamins A and C.

The addition of lactose, magnesium sulphate or mineral oil to the polished rice diet of the pigeons of group D, had no influence on the weight, respiration or temperature curves, and only a questionable effect on the usual blood sugar curve; however, in a large majority of the experiments, red blood counts as well as the hemoglobin levels were maintained within the normal limits throughout the polished rice feeding period; in other words, these agents prevent the rice anemia.

The beneficial action of magnesium sulphate or lactose on the rice anemia

is not the consequence of a change in the blood concentration at the time the observations were taken, since no noticeable changes have been observed either in the usual blood sugar levels or in our preliminary data on the blood volume or specific gravity, under similar conditions.

It is believed that the beneficial effects observed in rice disease as reflected by the red count and hemoglobin levels following the administration of mineral oil, magnesium sulphate or lactose is explained by the resultant catharsis with a consequent limitation of a possible intestinal bacteremia. The latter hypothesis, however, remains to be proven. It is in harmony with the observations of Beach and Davis (1925) to the effect that the feeding of lactose or dry skim milk afforded a considerable degree of protection to chicks which were artificially infected with Eimeria avium; they state that the degree of cecal acidity developed may be sufficient to destroy the sporozoite or merozoite forms of the infective agent.

SUMMARY

1. The administration of loosely bound hydrochloric acid (betaine HCl) or butter and orange juice does not prevent the depressive effects of rice disease on body weight, respiration, temperature and red corpuscle counts. These effects parallel those of partial starvation.

2. The administration of lactose, mineral oil or magnesium sulphate appeared to prevent the anemia without affecting the other depressive effects.

3. The beneficial action of lactose, mineral oil and magnesium sulphate is perhaps explained by their action on the intestinal bacterial flora and consequent diminution of bacteremia, and possibly by stimulation of peristalsis through a saline cathartic action.

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EXPERIMENTAL CRETINISM

II. The Influence of the Thyroid Gland on the Production and Control of Experimental Rickets[1]

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In a recent paper on experimental cretinism (Kunde and Carlson, 1927), it was demonstrated that thyroidectomized rabbits showing signs of hypothyroidism during the growth period manifest a defective calcification and growth of the bones simulating clinical rickets. This ricket-like condition is entirely independent of dietary factors inasmuch as it develops in a marked degree in cretin rabbits living on a dietary adequate in every way for growth and the rearing of young under normal conditions. The question naturally arises whether it would be possible to prevent the development of rickets in the cretin condition by adding an excess of the antirachitic vitamines to the dietary.

Since the rabbit is strictly an herbivorous animal with a somewhat restricted and well-defined dietary, it seems unphysiological to add substances of animal origin to its diet. The white rat was accordingly selected as the animal of choice for this experiment inasmuch as it is an omnivorous animal, the normal dietary habits being known as well as the ricket producing diets and the amount of cod liver oil necessary to prevent the development of rickets on established ricket-producing diets.

Experimental. Forty-six white rats from 6 different litters of known laboratory stock were selected for experimental purposes. Between 15 and 29 days after birth the rats of each litter were divided into 5 groups. The group differentiations were based on dietary factors and experimental conditions depending on whether or not the animals were thyroidectomized or normal. Groups A and B received a dietary consisting of table scraps of the various kinds of food which constitute the dietary of well-nourished individuals. This table scrap dietary contained an abundance of antirachitic vitamines. Group A was made up of normal rats; group B was thyroidectomized. Groups C and D each received the Steenbock and Black (1925) ricket-producing diet no. 2965. Cod liver oil was added to this diet in amounts varying from 2 per cent of the diet up to a quantity

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where food became objectionable. Group C were normal animals; group D, thyroidectomized. Group E was made up of normal animals receiving the Steenbock and Black diet no. 2965, without cod liver oil. The purpose of this group was to produce rickets for use in a comparative study of the histology of the epiphyses of the bones of the various groups.

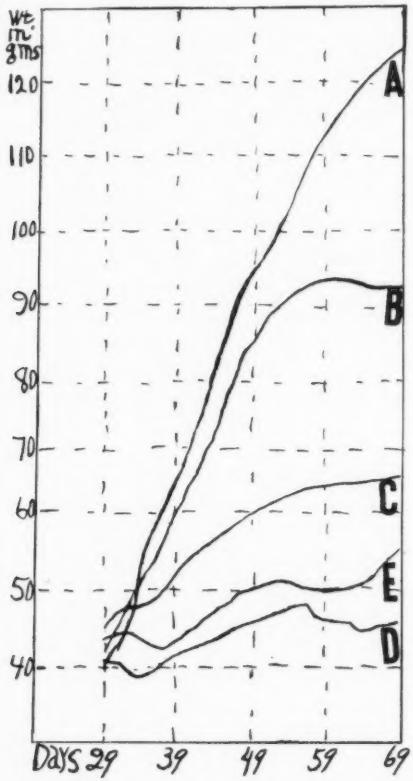

Fig. 1. Showing the growth curve of 5 rats (litter mates, litter II) A and B received a table scrap dietary containing foods rich in antirachitic vitamines. *B* = thyroidectomized at 29 days after birth; *A* = normal; C and D each received the ricket-producing diet plus cod liver oil. *D* = thyroidectomized 29 days after birth; *C* = normal; *E* = normal rat receiving the rachitic diet without cod liver oil.

The technique of the thyroidectomy is similar to that used in thyroidectomizing rabbits (Kunde and Carlson, 1927) with these exceptions: All rats were thyroidectomized between 15 and 29 days after birth, the parathyroid glands were located by means of a large hand lens as two salmon

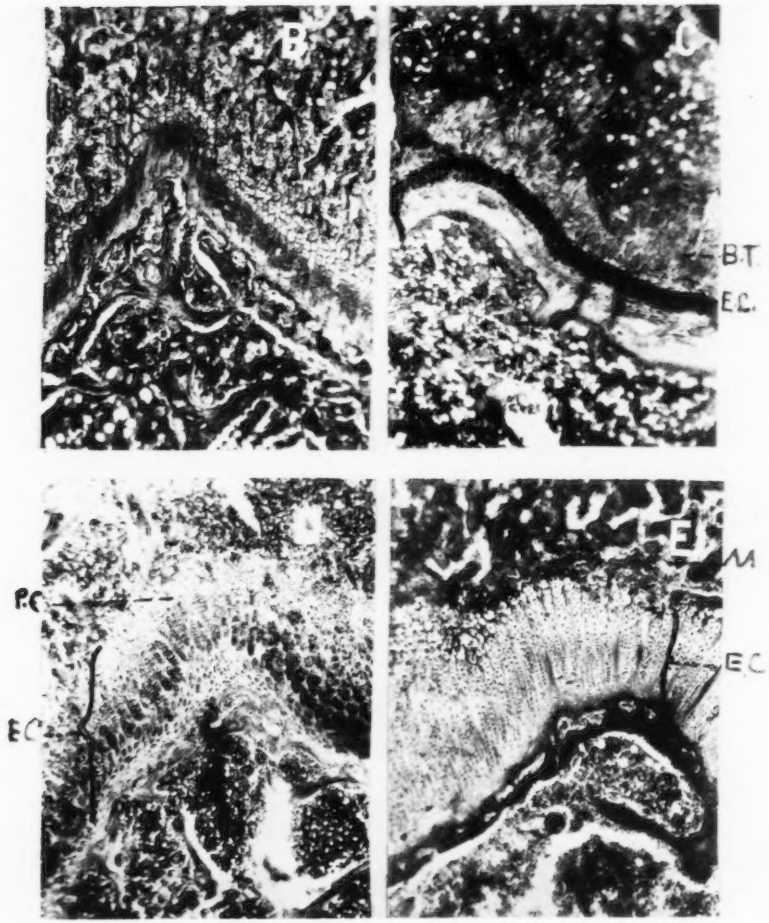

Fig. 2. Showing photomicrographs through the distal ends of the femurs. B, C, D and E are litter mates 69 days old (litter II); *B* = thyroidectomized rat on table scrap diet, mild rickets is present here. C and D received the rachitic producing diet plus cod liver oil. *D* = thyroidectomized, note the increased width of the epiphyseal disc of cartilage, *E.C.* and the irregular arrangement of the wide proliferating zone of cartilage, *P.C.*, which is entirely devoid of calcium; this is advanced rickets. *C* = the litter mate, with thyroid gland intact, here the epiphyseal disc, *E.C.*, is very narrow and completely calcified showing osteoporosis but no rickets. *B.T.*, normal bone trabeculae. *E* = unoperated litter-mate on rachitic diet only, showing far advanced rickets. Note the marked increase in the epiphyseal disc of cartilage which is entirely devoid of calcium. The metaphysis, *M*, consists of osteoid tissue separated by very vascular medullary areas. Staining = hematoxylin and eosin. Magnification = ×52.

pink spots near the superior poles of the lobes of the thyroid gland. These were carefully removed from the thyroid by means of blunt dissection with a fine dental probe, care being taken to leave their blood supply intact during the removal of the thyroid gland. Calcium lactate was given to the operated rats for not more than two days following the operation for the purpose of controlling temporary parathyroid deficiency which may have resulted from operative trauma to the parathyroids. In some instances it was impossible to spare the parathyroids due to anatomical anomalies. In these cases the animals developed severe tetany and died 4 to 24 hours after the operation. All animals were weighed twice weekly. The experiments terminated on the 69th day after birth. At this time the animals were killed and histological sections of the distal ends of the femurs through the epiphyseal region were made.

Results. The collective results of these experiments can be presented briefly by giving in detail the findings for litter II; figure 1 shows the growth curve of five litter mates segregated into the different groups on the 29th day after birth. The rats in groups B and D were thyroidectomized at this time. The dwarfing in growth of B due to removal of the thyroids is apparent by the characteristic flattening of its growth curve as compared to the curve of A. The two animals each received the table scrap diet. The difference in growth between C and D is even more strikingly apparent. Here each received the ricket producing diet of Steenbock and Black plus cod liver oil in quantities increasing in amount from 2 per cent of the diet up to a point where food became objectionable. From the curve it can be seen that the growth of D is even less than of E which received the rachitic diet with no cod liver oil but had its thyroid glands intact.

Histological sections through the epiphyseal region near the distal end of the femur of the rats in different groups are shown in figure 2. Advanced rickets is apparent in D, and milk rickets in B. These animals were both thyroidectomized, B received the same dietary as A, i.e., table scraps which contained an abundance of antirachitic substances in the butter fat, green vegetables and cereals. But no amount of dietary precautions were sufficient to prevent the occurrence of mild rickets in the thyroidectomized rat. D received the ricket producing diet plus an abundance of cod liver oil. Histological study of the epiphysis shows advanced rickets, indicating that no amount of cod liver oil added to the ricket producing diet of these thyroidectomized rats was sufficient to prevent the occurrence of a marked disturbance in the calcification and a wide zone of proliferative cartilage in the epiphyseal region. Figure 2 C shows the epiphysis of the unthyroidectomized litter mate which received the same dietary as D. Here the epiphyseal disk of cartilage is narrow with an even margin and calcification is complete.

Our results show that no amount of cod liver oil added to a ricket-pro-

ducing diet can prevent the occurence of rickets in the thyroidectomized rat as determined by histological studies of the epiphysis at the distal end of the femur. Moreover, no amount of food substances containing an abundant amount of antirachitic vitamines added to the diet is capable of preventing the development of mild rickets in the rat thyroidectomized between 15 to 29 days after birth. These rats were not exposed to the unfiltered rays of the sun.

SUMMARY

No amount of cod liver oil added to a ricket-producing diet is adequate to prevent the development of rickets in cretin rats as determined from histological studies of the epiphysis.

In the cretin rat, supplying antirachitic vitamines in the form of vitamine-rich foodstuffs to a nutritious diet consisting of table scraps, cannot prevent the occurrence of rickets.

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DIFFERENTIAL METABOLISM IN BRAIN TISSUE AS INDICATED BY LACTIC ACID DETERMINATIONS

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Brain tissue has been shown to have an acid metabolism similar to that which occurs in muscle (1). Under conditions of oxygen deficiency lactic acid accumulates in the tissue and when there is an extreme want of oxygen the accumulation of lactic acid is extremely rapid. McGinty and Gesell (1) proved that lactic acid is formed in the entire brain under anaerobic conditions and concluded from their experimental studies that there is an acid metabolism of the respiratory center. Their experiments however do not prove that the *rate* of metabolism in the respiratory center is the same as that in other parts of the brain. The differential water absorption by four different portions of brain substance which may be attributed to differences in chemical composition (2) suggest that there may also occur in the brain a differential metabolism. The present paper is a report of a comparative study of the rate of metabolism in the four parts of the brain—the cerebral hemispheres, cerebellum, midbrain and medulla.

EXPERIMENTAL METHODS. Rabbits were used throughout the experiment. The animal was decapitated and, with one blow of a hammer on a sharp knife which was held over the mid-line of the head, the skull and brain were split in half. In most instances the brain was symmetrically divided into portions approximately equal. One-half of the brain was removed from its skull encasement, the cerebral hemispheres, cerebellum, midbrain and medulla separated and dropped into liquid air which froze the brain tissue immediately. By this method the tissue is obtained as near to the normal as possible with regard to lactic acid content. On the average one minute elapsed from the moment of decapitation until the brain substance was in liquid air. When the entire brain is placed in liquid air it is frozen so hard that it is difficult, and oftentimes impossible, to separate the different parts of the brain. Consequently, although a few seconds more were required, it was found more satisfactory, first to separate the brain portions and then to drop each one into an individual beaker containing liquid air. The second half of the brain in its skull encasement was placed immediately after decapitation in a vacuum jar which was

standing in an incubator at 37°C. The temperature was kept constant throughout the experiment. The jar was evacuated and the half-brain allowed to remain in the vacuum for various lengths of time as designated in the tables. When removed from the skull it was separated into four parts and dropped into liquid air in the same manner as the first half of the brain. In all instances time was recorded with a stopwatch.

Each portion of the brain substance was removed from the liquid air after it had been frozen solid, weighed promptly and then ground up in a mortar with sand to a thin brei. To each portion 5 to 10 cc. of 10 per cent Na_2WO_4 and $\frac{2}{3}$ normal H_2SO_4 were added. Larger amounts of tungstate and acid were used for the cerebral hemispheres than for the smaller portions of brain substance. In some instances, owing to the rapidity with which it was necessary to remove and divide the brain and to the difficulty of making a perfect division, the entire half of the various portions, particularly of the medulla, could not be obtained. Lactic acid determinations on a sample of tissue however gave results that were concordant with those obtained from an entire half of the brain portion.

The contents of the mortar containing the tungstic acid precipitate were poured into a flask and to this was added the water with which the mortar was rinsed. Calcium hydroxide and copper sulfate solution were added to remove the sugar (3) and the mixture centrifugalized. The supernatant liquid was analyzed for lactic acid by the procedure of Clausen (4). Pure zinc lactate was prepared and recrystallized from water four times and a sample was obtained which was 99.98 per cent $Zn(C_3H_5O_3)3H_2O$, as indicated by igniting and weighing it as zinc oxide. This sample was used as the source of lactic acid in standardizing this method. The standard lactic acid was oxidized to acetaldehyde and the latter titrated according to the method of Ripper (4).

Consistent results could not be obtained when potassium permanganate was used as the oxidizing agent. When 50 per cent sulfuric acid solution was used, the amount of aldehyde recovered was consistently around 98.5 per cent, a value somewhat higher than that given by Clausen. The deviation was less than 0.6 per cent when the lactic acid content varied from 0.5 to 10 mgm.

After removal of the glucose by the addition of calcium hydroxide and copper sulfate, an aliquot portion of each sample was oxidized in the reaction tube. The aldehyde was carried over from the reaction tube by a warm current of air and led into sodium bisulfite solution. The excess of the bisulfite solution was released by sodium bicarbonate and titrated with standard iodine solution, from which data the lactic acid content of each sample was calculated. The determinations on the four samples were made simultaneously. The results are given in the tables.

Results and conclusion. The data presented in the tables show that

under anaerobic conditions lactic acid is formed in brain tissue but not to the same extent in the four parts of the brain; viz., cerebral hemispheres, cerebellum, midbrain and medulla. The least amount per unit weight of tissue occurs in the cerebral hemispheres, the percentage content increasing in the order: cerebellum, midbrain, medulla. Medulla tissue contains more than twice the amount of that found in cerebral hemisphere tissue.

TABLE 1

RABBIT BRAIN (FIRST HALF)	TIME INTERVAL BETWEEN DECAPITATION AND FREEZING	LACTIC ACID CONTENT—MGM. PER 100 GRAMS TISSUE			
		Cerebral hemispheres	Cerebellum	Midbrain	Medulla
	seconds				
1	60	14.7	20.2	29.6	47.5
2	70	11.6	18.1	26.3	37.2
3	53	15.9	33.8	43.7	80.0
4	60	14.7	19.5	20.0	20.3
5	67	12.1	18.5	34.3	
6	70	13.1	19.2	32.1	39.0
7	75	11.5	19.5	18.7	20.9
8	75	13.0	16.7	25.7	47.0
9	78	13.8	20.5	28.8	27.0
10	80	15.0	24.0	25.4	

TABLE 2

RABBIT BRAIN (SECOND HALF)	TIME INTERVAL BETWEEN DECAPITATION AND FREEZING	LACTIC ACID CONTENT—MGM. PER 100 GRAMS TISSUE			
		Cerebral hemispheres	Cerebellum	Midbrain	Medulla
	seconds				
1	300	19.2	23.9	30.3	53.5
2	300	15.7	28.0	21.9	53.5
3	145	16.9	40.5	45.5	88.0
4	300	18.8	24.4	21.8	23.8
5	480	15.9	22.5	37.0	51.4
6	150	16.1	22.6	33.8	43.4
7	480	14.5	23.6	19.9	23.8
8	240	16.9	20.8	28.0	56.0
9	240	17.8	25.2	31.0	31.6
10	800	19.7	29.8	28.4	

After various time intervals ranging from 145 to 800 seconds, the lactic acid content increases in the four parts of the brain substance, the order of percentage content however remains the same, the least amount in the cerebral hemispheres, the greatest in the medulla. From our data we are unable to plot time curves, but the data suffice to show that there is an acid metabolism in each of the four portions of the brain substance, and

that at any given moment, under anaerobic conditions, there is a greater per cent of lactic acid present in the medulla than in the other portions and the least percentage content in the cerebral hemispheres. From these results it is concluded that acid metabolism occurs at different rates in various portions of brain tissue.

SUMMARY

Previous investigations have shown that there occurs in brain tissue an acid metabolism.

1. Under anaerobic conditions the lactic acid content of different portions of brain tissue of the rabbit after various intervals of time has been determined.

2. The percentage content of lactic acid is different in the four portions of the brain: cerebral hemispheres, cerebellum, midbrain and medulla.

3. The percentage content increases in the following order: cerebral hemispheres, cerebellum, midbrain, medulla. The least amount per unit weight occurs in the cerebral hemispheres, the greatest in the medulla.

4. More than twice as much lactic acid is formed in a unit weight of medulla tissue than in the tissue of the cerebral hemispheres.

5. It is concluded from the data presented that the rate of acid metabolism is different in various portions of brain substance.

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A STUDY OF THE EFFECT OF MEASURED DOSES OF ULTRA-VIOLET RADIATION ON THE IRRITABILITY OF MELANOPHORES

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The melanophores, in the scales of fish, contract on stimulation and their reactions are regulated entirely by the nervous system in the living fish. When the scales are removed from the fish and placed in isotonic Ringer's solution the epidermis becomes permeable where it is torn, and the melanophores react toward various chemical and physical agents. The large body of work published on the subject of melanophores deals, for the most part, with their behavior in the living fish while under the control of the nervous system. Spaeth, however, published a series of papers in which he studied the behavior of the melanophores in scales removed from the fish and placed in isotonic salt solutions and, as these experiments follow his technique, his results will be briefly summarized.

Spaeth found (1913) that melanophores contract in 0.1 M KCl or 0.1 M $CaCl_2$ and expand in 0.1 M NaCl. The sodium chloride represents a lack of exciting stimulus. The contraction in KCl and $CaCl_2$ is rapid, but reversible if the scales are returned to 0.1 M NaCl. In an isotonic Ringer's solution there is a balanced condition of partial contraction. The pigment cells contract reversibly when stimulated by an induction current, either in sodium chloride or Ringer's solution, and they contract also on exposure to ultra-violet light although they show no reaction to visible light. Spaeth believed that, under these conditions, the contracting stimuli act directly on the cells and not on the cells through the torn nerve endings. He came to this conclusion because the contraction in salt solutions begins at the edge of the scale and works in and the contraction in ultra-violet light begins at the centre where the epithelium is thinnest, and the intensity therefore greatest, and works out. If these stimuli acted on the torn nerve endings there would be a synchronous reaction in the whole group of melanophores. The melanophore in a separated scale seems, therefore, to offer an excellent opportunity for the study of the reactions of an isolated living cell to measured doses of ultra-violet radiation.

Spaeth, in considering the mechanism of the reaction, suggested that

the melanophore may consist of a colloidal suspension of melanin granules which aggregate on contraction and disperse on expansion. According to this view the contraction and expansion would represent a reversible coagulation and it seemed probable that further investigation of the action of ultra-violet radiation on melanophores might furnish proof for or against this theory of the mechanism of contraction.

PURPOSE OF THE INVESTIGATION. This study was undertaken with the following objectives:

1. To determine the amount of ultra-violet energy necessary to produce increase in irritability, decrease in irritability, and lethal action in isolated living cells.

2. To make a quantitative comparison of the effect of ultra-violet radiation with and without visible light and infra-red rays. Some investigators have come to the conclusion that visible light inhibits the action of ultra-violet radiation. Harris (1925) has recently published a paper in which he concludes that the stimulating action of ultra-violet radiation on the gaseous metabolism of small animals and on the movements of the isolated stomach of the frog is completely annulled by the presence of visible radiations. On the other hand, Azuma and Hill (1926) have repeated the experiments of Harris on involuntary muscle, and have shown that no physiological interference exists between visible and ultra-violet radiations. The reaction of melanophores to radiation seemed to offer an excellent method for investigating this disputed point.

3. To investigate the mechanism of contraction in the melanophore. In view of the known effects of ultra-violet light on colloidal suspensions it was hoped that further study of the light reaction would give some evidence for or against Spaeth's theory that melanophore contraction is of the nature of a colloidal aggregation.

METHOD. Scales taken from *Fundulus heteroclitus* were used as a source of the melanophores in all the experiments reported. The fish were washed in distilled water, and the scales were then carefully removed and placed in an isotonic Ringer's solution, made up according to the formula used by Spaeth. The scales were left in this solution for varying lengths of time and transferred as desired to the other solutions. In the great majority of experiments, and unless otherwise stated, the scales were placed in 0.1 M NaCl after a five minute interval in the Ringer's solution, as the expanded state was found to be the most satisfactory one for experimentation. Between each transference the scales were drained on filter paper before being placed in the fresh solution. It was necessary to exercise care in the handling of the scales, and to touch them only on the bony part as the melanophores contract when mechanical stimuli are applied as readily as with chemical stimuli.

Stimulating mechanism. The rate of contraction of melanophores in

response to electrical stimulation was used as a measure of their irritability in most of the experiments. For this purpose a stimulating apparatus was used, copied from that designed by Spaeth (1916 a) (see fig. 1).

Three ordinary dry cells were used as a source of current, and these were

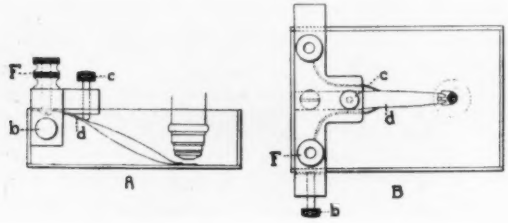

Fig. 1. *A*, side view of apparatus; *B*, top view of apparatus; *F*, binding post; *b*, horizontal set screw; *c*, vertical set screw; *d*, free end of thin strip of spring brass with platinum electrodes attached (from Spaeth).

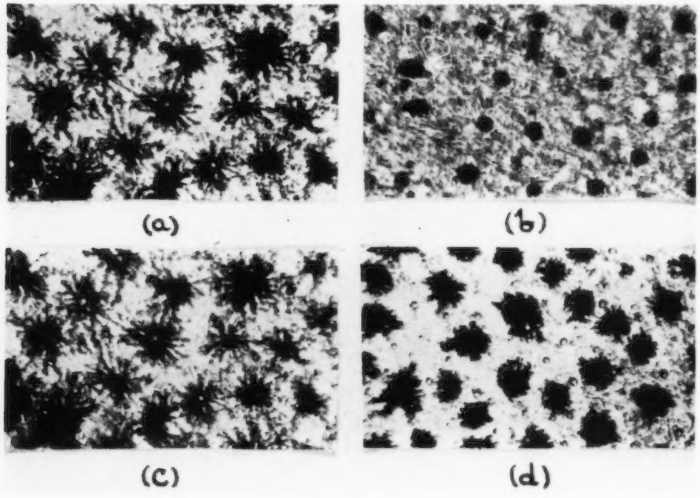

Fig. 2

connected with an inductorium having a switch key in the primary circuit. A tetanizing current was used throughout.

All experiments were performed at room temperature except one series with 0.1 M $CaCl_2$ and 0.1 M KCl at 0°C.

In figure 2 photographs are shown of melanophores in a condition of

a, complete expansion; *b*, complete contraction; *c*, progressive contraction, and *d*, partial contraction.

Radiation. A Cooper-Hewitt quartz mercury vapor air-cooled Lab-arc was used for the ultra-violet radiation. All experiments were performed with the arc at the same angle and exactly five inches from the scale that was being radiated. The arc was allowed to burn ten minutes before the beginning of radiation to insure steady conditions. The scales were placed in the 0.1 M sodium chloride solution in a depression slide,

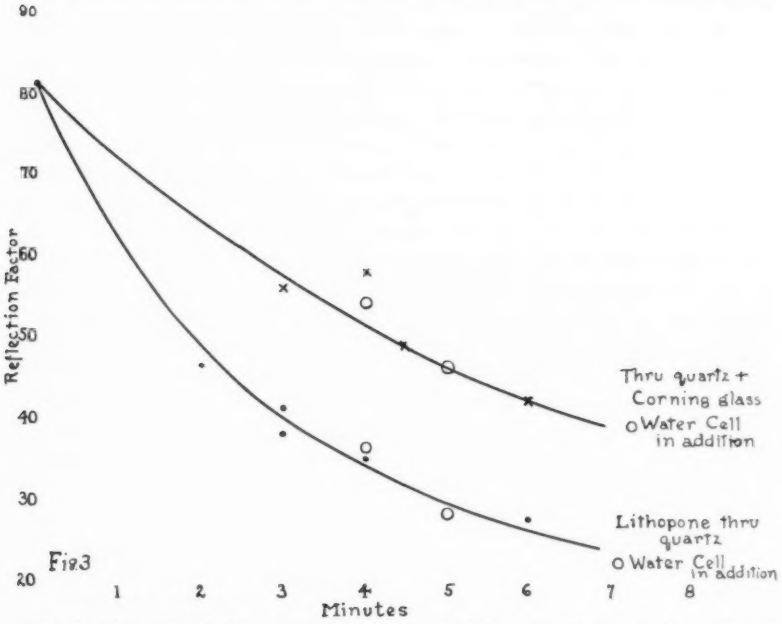

Fig. 3. Lithopone darkening on exposure to the Lab-arc at 5 inches, after the arc has been running 10 minutes.

The time necessary to give one lithopone unit of ultra-violet energy is the time necessary to darken lithopone to a reflection factor of 50 per cent. One lithopone unit is given by the bare arc in 2 minutes, and by the arc filtered through Corning glass G986A in 4.5 minutes.

and were not moved from the stage of the microscope in order that the identical scale and definite melanophores could be observed. On account of the position of the microscope it was necessary to radiate at a small angle.

Careful lithopone measurements were made according to the method devised by Clark (1924). With this method the time required by the arc to darken lithopone paint to a reflection factor of 50 per cent is called one lithopone unit (L. U.) Using this measurement, the Lab-arc alone or the Lab-arc plus the quartz water cell was found to give one lithopone

unit of radiation in two minutes at five inches. With Corning glass G986A, 3 mm. thick, which only transmits rays of wave-length 250–400 mμ, as a filter, it took 4.5 minutes to give one lithopone unit at this distance. The ultra-violet intensity was, therefore, 2.25 times as great with the Lab-arc alone, or plus the quartz water cell, as with the Lab-arc filtered through Corning glass. The Corning glass was used to screen out all visible rays, and the water cell to screen out the infra-red in an effort to determine whether or not these rays interfered with the action of the ultra-violet rays.

Lithopone unit. Figure 3 shows the method by which the lithopone unit was obtained. Lithopone, in water paste, was pressed between quartz plates, and the reflection factor of the surface was tested by means of a Macbeth illuminometer. It was found to be 82 per cent. It was then exposed to the Lab-arc at five inches, in the position to be occupied by the scale. After a two-minute exposure the reflection factor was found to be 50 per cent, and after a four-minute exposure 35 per cent. When a number of determinations had been made a curve was drawn for reflection factor against exposure time, and the time necessary to darken lithopone to a 50 per cent reflection factor was taken from the curve. This time represents one lithopone unit of ultra-violet energy. The sensitivity curve of lithopone shows that it begins to darken at about 320mμ, and that the sensitivity increases sharply at 300mμ, and is fairly constant from 300mμ down to the limit of transmission of quartz.

The method is similar to measurements of intensity with a photographic plate, but the lithopone reacts only to wave-lengths shorter than 320mμ, which are just the wavelengths that are effective in producing physiological light reactions.

Lithopone, therefore, gives a convenient method for measuring the amount of physiologically effective ultra-violet radiation in a given source of light and Clark's work (1924) has shown very good agreement between the energy, as measured in lithopone units, and the physiological effects produced by artificial sources of ultra-violet light.

Results. *Ultra-violet radiation alone.* Radiation of *Fundulus* scales, lasting from one minute to one hour, gave some contraction, but the degree of response, as measured by the amount of contraction, varied from scale to scale, and among the different melanophores of one scale, to such an extent that no fixed point could be chosen as a basis of comparison.

Observations made thirty minutes and one hour after radiation showed that the ultra-violet contraction is irreversible in 0.1 M NaCl solution, and increases somewhat in extent for some time after cessation of the stimulus. When the scales were exposed in isotonic Ringer's solution it was found that melanophores which had received any amount of radiation, up to forty minutes, expanded completely when placed in 0.1 M NaCl solution after radiation.

It was thought that, owing to the presence of calcium, the scales might show more complete contraction, when exposed in Ringer's solution, than when given the same exposure in sodium chloride, but this was not the case.

Lethal dose. After a radiation of fifty minutes, in Ringer's solution, the melanophores were partially contracted, and only expanded very slightly after ten minutes in 0.1 M NaCl. After a radiation of fifty-five or sixty minutes the melanophores, though only partially contracted, showed no expansion on being transferred to 0.1 M NaCl. Melanophores, radiated in 0.1 M NaCl showed no contraction on electrical stimulation after a radiation of fifty-five minutes. Therefore a radiation of fifty-five minutes, at five inches from the Lab-arc, which is equivalent to 27.5 lithopone units of ultra-violet energy, was found to be the lethal dose.

Radiation plus electrical stimulus. Summation of stimuli. With the electrical stimulus alone, the cells expand when the stimulus is removed, the expansion requiring about ten minutes, as a maximum, in a normal scale.

With the ultra-violet radiation alone in 0.1 M NaCl there is an immediate partial contraction which sometimes is greater in half an hour, and which usually shows no further change after an hour irrespective of duration of radiation.

With electrical stimulation after ultra-violet radiation there is complete contraction from which there is recovery to the point of contraction due to light alone.

With electrical stimulation before ultra-violet radiation the sensitivity to ultra-violet light is diminished and it takes more radiation than normal to produce contraction in a scale. This does not seem to be due to a refractory period since a scale that has been contracted by an electrical stimulus contracts just as rapidly, or even more rapidly, when a second electrical stimulus is applied immediately after expansion.

Change in irritability after radiation. With electrical stimulation before and after radiation, the irritability of the cells shows an increase for small amounts of radiation, and for longer radiation there is a decrease in irritability and eventually death. Measurements were made of the time necessary to give complete contraction, on electrical stimulation, before and after radiation. The per cent change in time was then calculated and taken as a measure of change in irritability. A faster contraction rate after radiation indicates increased irritability. The first cells in a scale that contracted completely were used as the standard in making these determinations. The melanophores that thus responded to the electrical stimulus were carefully located, and the same ones observed for the electrical stimulation following radiation.

The following curve (fig. 4) in which per cent change in contraction

time is plotted against time of radiation, shows the effect of different amounts of radiation upon the irritability of the melanophores to electrical stimulation. This curve shows very definitely that the initial effect of ultra-violet light is to heighten the irritability and that this effect increases with the dose up to a certain point.

Beyond this point the increase in irritability becomes steadily less, although the contraction time is still more rapid than normal. After a

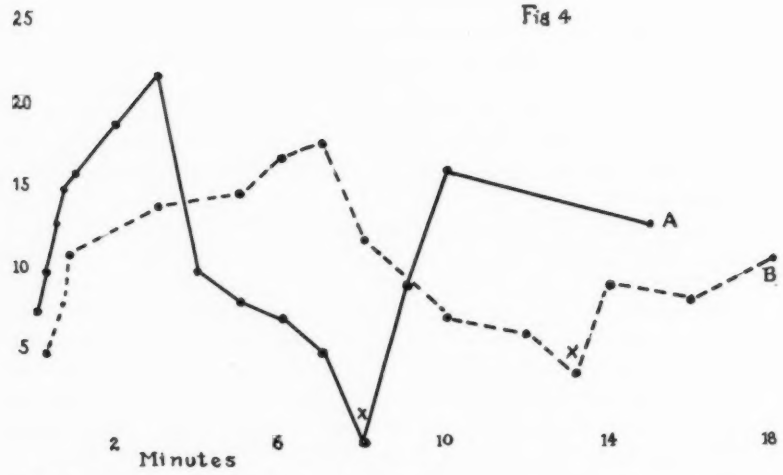

Fig. 4. Abscissae: Radiation time in minutes.

Ordinates: Per cent decrease in contraction time. The first rise indicates increased irritability. At *X* light contraction begins and brings about the second rise. The rate of electrical contraction is then more rapid because the cells start from a state of partial contraction.

radiation of 8 minutes, with the bare arc, the increase in irritability has disappeared, and the contraction time before and after radiation is the same. With longer radiations there would probably be a decrease in irritability, but at this point the melanophores begin to show a light contraction. The rate of electrical contraction then becomes more rapid because the cells instead of being completely expanded, start from a state of partial contraction. This brings about the second rise in the curve which does not indicate a second increase in irritability.

The second decline is due to the injury done by the light which becomes so great, for radiations greater than 20 minutes, that the melanophores fail to show complete contraction on electrical stimulation. After a radiation of 55 minutes they do not respond to the electrical stimulus at all and are presumably dead.

It seems probable that this observation may have a bearing upon the therapeutical applications of ultra-violet light, since it indicates that the

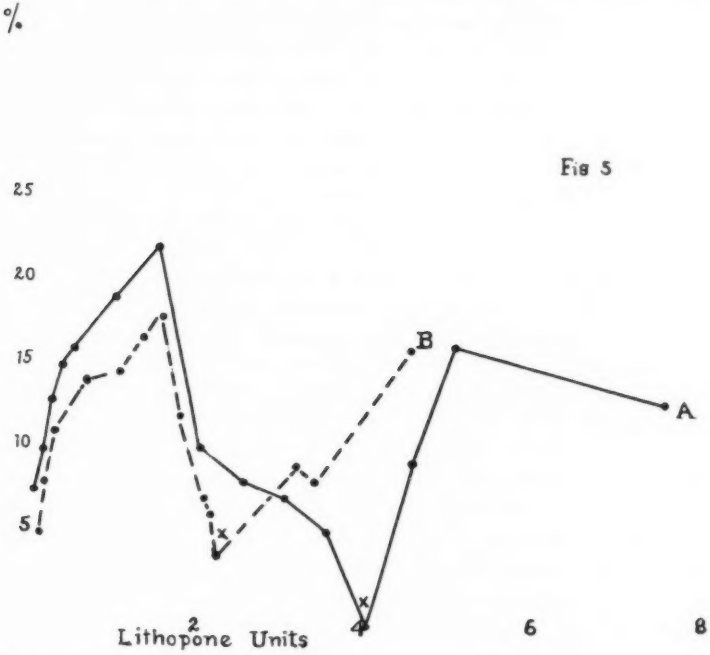

Fig. 5. Abscissae: Energy of radiation in lithopone units.
Ordinates: Per cent decrease in contraction time. The first rise indicates increased irritability. At *X* light contraction begins and brings about the second rise.

irritability of a cell may be increased or decreased or destroyed according to the dosage of radiation, and it is to be noted that the maximum beneficial effect, on the melanophore at least, is obtained with a short exposure.

Curve *B*, figure 4, is an illustration of the same type of experiment with the Corning glass G986A used as a screen. This shows that the type of effect produced is the same through the Corning glass and with the bare arc, but that since the intensity is reduced it takes a longer time to get the same effect.

In figure 5, per cent change in contraction time is plotted against lithopone units of radiation. Figure 5 shows maximum increase in irritability through Corning glass G986A after 7 minutes of radiation which is equivalent to 1.55 L. U., while with the unfiltered quartz arc there is a maximum increase in irritability after 3 minutes of radiation which is equivalent to 1.5 L. U.

This very close agreement shows that, if the lithopone unit is a satisfactory measurement for the intensity of the biologically effective ultra-violet light given off by quartz Hg arcs, then the same effect can be obtained with the same amount of ultra-violet radiation when visible light is cut out. Since screens such as Corning G986A, which cut out the visible, also cut down the intensity of the ultra-violet, the radiation time must be lengthened to give an equivalent amount of light before an equivalent effect is obtained. Cases in which observers, such as Harris (1925), have reported stimulation with ultra-violet radiation alone, and no stimulation with ultra-violet and visible light may easily be explained in light of the results given in figures 4 and 5. It was probable that in his case the ultra-violet energy was equal to a stimulating dose when the uviol screen, absorbing the visible rays, was used but that, on removing the screen to add visible light, the intensity of ultra-violet radiation was increased also, and thus became too great to give a stimulating dose, and gave an inhibiting dose instead. From figure 4 one can see that a seven minute radiation with the Corning G986A screen gives maximum increase in sensitivity for melanophores and with the bare arc seven minutes gives practically no stimulation at all. In terms of time this would suggest greater activity for the ultra-violet radiation minus the visible, which is an erroneous conclusion. In terms of ultra-violet energy (fig. 5) maximum stimulation is given by a certain definite dose of ultra-violet energy whether visible rays are present or not.

The fact that there is an increase in irritability in melanophores for small doses of ultra-violet light and with larger doses a decrease in irritability, and eventually death, agrees very well with the results of Peña and Clark (1924) on the effect of ultra-violet light on pathogenic fungi. They found that cultures of a pigmented fungus, *Montoyela niger*, exposed to a large Hanovia quartz Hg arc, grew faster than the controls for exposures of 1 to 10 minutes, the greatest stimulus to growth occurring after an exposure of 4 minutes which, under their conditions, amounted to 4.4 L. U. For exposures greater than ten minutes the growth was less than normal and a lethal effect was produced by an exposure of fifty-five minutes = 61 L. U.

Since both the fungus and the melanophore are pigmented cells, a comparison of their respective stimulating and lethal doses is very suggestive.

Mechanism of the reaction. It was hoped that further studies of the reaction of melanophores to ultra-violet radiation would throw some light

on the mechanism of contraction. If the contraction is a reversible colloidal coagulation, as suggested by Spaeth, then one would expect the reaction to ultra-violet radiation to be different at different values of hydrogen ion concentration. Clark (1925) has shown that salt-free egg albumin coagulates, with ultra-violet light, only when radiated at a pH near the isoelectric point, or if brought to this pH after radiation. If the light contraction in a melanophore is a coagulation of melanin granules one would expect to find some range of pH, near the isoelectric point, where contraction would be more rapid and more complete than normal.

As no previous work had been done on the contraction of pigment cells at different hydrogen ion concentrations, solutions of 0.1 M NaCl were brought to pH 6.8, 6.2, 5.0 and 4.2 by means of dilute HCl, the pH of the sodium chloride being normally about 7.6. Sodium chloride solutions were also brought to the same hydrogen ion concentrations with dilute acetic and lactic acid. The results were in general the same with all three acids so that only the results with the HCl solutions are given.

TABLE 1

Electrical stimulus alone immediately after scales are placed in solutions

pH	AVERAGE CONTRACTION TIME	AVERAGE TIME OF EXPANSION
7.6	46 seconds	2 minutes 27 seconds
6.8	49 seconds	1 minute 55 seconds
6.2	37 seconds	1 minute 57 seconds
5.0	49 seconds	1 minute 55 seconds
4.2	42 seconds	1 minute 50 seconds

Electrical contraction in solutions of different pH. The time of contraction and expansion after an electrical stimulus was measured and the results are given in table 1. There is apparently no difference in contraction time after electrical stimulus in solutions of different pH when the scales have been in the solutions for the minimum amount of time. The expansion time is slightly slower in pH 7.6 than in neutral or slightly acid solutions, while the rate of contraction is greatest at 6.2.

After the scales have remained in the acidified solutions for one hour the rate of contraction is more variable, because in most of the solutions the acid itself causes a slight contraction. However, the variations are so slight that one must conclude that there is no very marked difference in the rate of electrical contraction in 0.1 M sodium chloride solutions of pH 4.0 → 7.8.

Electrical contraction and radiation combined in solutions of different pH. As a radiation of 3 minutes had been found to give the maximum stimulation to melanophores in 0.1 M sodium chloride solution (pH 7.6 → 7.8)

it was decided to try the same type of experiment in solutions of different pH.

The results are represented graphically in figure 6. This figure shows that the increase in irritability which results from a three minute radiation

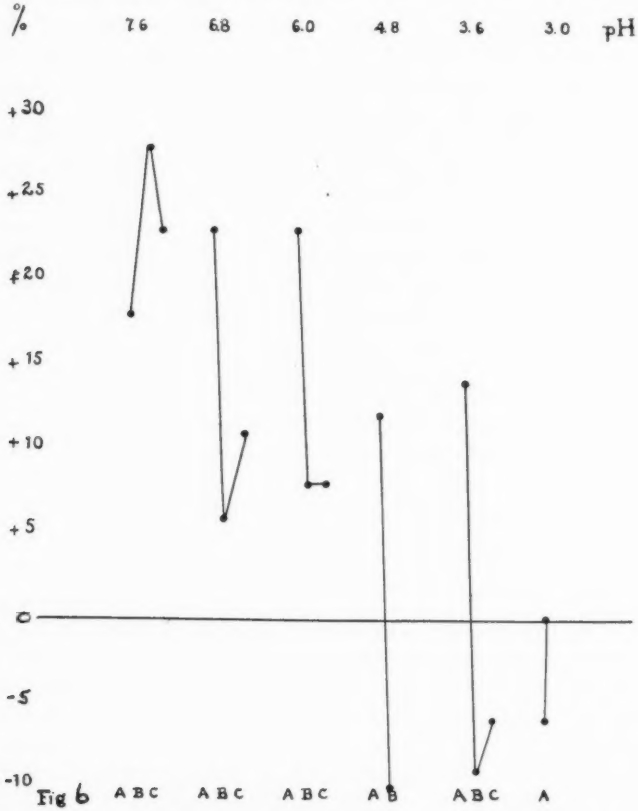

Fig. 6. Abscissae: pH. *A*, *B*, *C* denote observations taken immediately, *A*, 30 minutes *B*, and 1 hour *C*, after immersion in solutions acidified with HCl.

Ordinates: Per cent changes in contraction time (+indicates more rapid contraction, or increased irritability, − indicates slower contraction time, or decreased irritability).

with ultra-violet light, persists for an hour when the scales are radiated at pH 7.6. A normal increase in irritability follows radiation in pH 6.8 and 6.0, but this increase becomes progressively less in more acid solutions, and disappears at pH 3.0. The increase in irritability does not persist in acid

solutions, becoming less 30 minutes to one hour after radiation in pH 6.8 and 6.0 and changing to loss in irritability in pH 4.8 and 3.6. The increase in irritability, resulting from exposure to ultra-violet radiation is greatest in slightly alkaline solutions.

Radiation alone in solutions of different pH. After scales had been in solutions of different pH for 30 minutes they showed normal reactions to ultra-violet radiation alone. At every pH a 10-minute radiation produced a partial contraction.

If the contraction of a melanophore is due to a reversible colloid aggregation, as suggested by Spaeth, one would expect markedly different reactions to ultra-violet radiation in the region of an isoelectric point even though the presence of salts may confuse the results somewhat. As none of the experiments performed gave any evidence of an isoelectric point in the range of pH used, and as this is the range in which the isoelectric points of most colloids are found, the experiments do not support Spaeth's theory.

It is, of course, impossible to say what the pH of the melanophore itself may be when the scale is immersed in the different solutions used, but the results shown in figure 6, on the increase in irritability after radiation, indicate that after thirty minutes in solutions of different pH the acid has penetrated the scale sufficiently to alter the reaction of the melanophores.

Effect of ultra-violet radiation on permeability. Packard (1925) found that *Paramecia* showed increased permeability after exposure to ultra-violet radiation and Tchahotine observed that when a narrow pencil of ultra-violet rays is directed on a sea-urchin's egg, the point of illumination shows a greatly increased permeability to water.

These results suggest that the increase in irritability after a 3-minute radiation may be due to an increase in permeability of the cells. To see whether this is true or not, experiments were performed in which the rate of contraction of the same melanophores, in response to chemical agents, was measured before and after exposure to ultra-violet radiation.

The 0.1 M solutions of KCl and $CaCl_2$ cause such a rapid contraction that the rate cannot be measured. Therefore a solution was made of 10 cc. isotonic Ringer's solution with 1.5 cc. of 0.1 M KCl added. This was very satisfactory as the melanophores contracted slowly enough to be timed, and the contraction was readily reversible.

Scales with the melanophores fully expanded in 0.1 M NaCl were placed in this mixed solution and the contraction time taken. The scales were returned to the NaCl solution, and after expanding were radiated for 3 minutes. Immediately after radiation the scales were replaced in the same mixed solution, and the contraction time again taken. In every instance, but one, the time was slower, the average change in time being 42.3 per cent.

At the same time a series of controls was run, using the procedure as recorded above except that the scales were not radiated but left in the sodium chloride solution for the same length of time as the others. These, also, showed a slower contraction time, the change in rate being 22.5 per cent in contrast to the 42.3 per cent for the radiated melanophores.

These results indicate a decreased permeability after an amount of radiation that gives the greatest increase in irritability.

As previous experiments had shown (see fig. 4) that after a 7-minute radiation the increase in irritability had disappeared, and that slightly longer radiations gave a light contraction, a series of experiments was performed using the technique as just recorded, but radiating for 7 minutes. These cells, likewise, showed a slowing in contraction time of 32.2 per cent. There seems to be still a slight decrease in permeability at the point where radiation produces no increase in irritability, and light contraction is about to begin. It is possible that the increase in permeability found by Packard may occur after exposures of sufficient length to cause a light contraction. This could not be measured because once the light contraction begins, accurate measurements of contraction time can no longer be made that are comparable with measurements made from a state of complete expansion. But it seems clear from these experiments that the increase in irritability found after very short radiations with ultra-violet light cannot be due to an increase in permeability.

Comparison with muscle contraction. Spaeth (1916 b) came to the conclusion that the melanophore is a modified smooth muscle cell, the contracted phase corresponding physiologically to the contraction of smooth muscle. He based this conclusion on the fact that stimuli which cause contraction of smooth muscle also act upon the melanophore; for example, induction currents, 0.1 M KCl, pressure and heat at 30°C.

Since muscles lose their irritability at 0°C., or slightly below, a few experiments were tried on the effect of contracting stimuli at this temperature. When scales were placed in 0.1 M $CaCl_2$ at 0°C. there was a greater expansion with the formation of very pronounced vacuoles showing lack of response toward this chemical stimulus at 0°C.

When scales were placed in 0.1 M KCl there was complete contraction although it took place much more slowly than at room temperature.

The contraction time after electrical stimulation was then measured, using the same scales at 0°C. and at room temperature. The results given below in table 2 show a slower contraction time, or loss in irritability, at 0°C.

As far as they go, these experiments bear out Spaeth's hypothesis that melanophores act as modified smooth muscle cells, since they react in the same way to temperature changes.

The partial contraction following ultra-violet radiation seems to be

similar to an increase in tonus in muscle, and it is interesting to note that recent experiments by Azuma have shown an increase in tonus in involuntary muscle after radiation with ultra-violet light (1926).

Azuma, in his most recent paper, finds that skeletal muscle, as well as smooth muscle, shows an increase in tonus on radiation with ultra-violet light. His results with muscles run very parallel to the results obtained in this work with melanophores. He found that the excitability of a muscle, when exposed to ultra-violet rays, and going into contracture, becomes less and less, and he is inclined to regard the contracture as a kind of rigor mortis (1927). With melanophores there was an initial increase in excitability, but when contraction began there was a decrease in excitability ending in the death of a cell in a contracted condition. These parallel results with isolated cells and with the whole muscle, tend to confirm Adler's hypothesis (1920) that the contraction of muscles in ultra-

TABLE 2

Electrical stimulation alone. Scales in 0.1 M NaCl pH 7.6

CONTRACTION TIME ON ICE	CONTRACTION TIME AT ROOM TEMPERATURE
1 minute 40 seconds	0 minute 38 seconds
1 minute 25 seconds	0 minute 55 seconds
1 minute 25 seconds	0 minute 40 seconds
2 minutes 0 second	0 minute 35 seconds
1 minute 25 seconds	0 minute 40 seconds
Average 1 minute 35 seconds	Average 0 minute 41 seconds

violet light is due to changes in the muscle cell, and not to an effect on the nerves.

Azuma believes that calcium ions are necessary to produce contracture under ultra-violet radiation in spite of the fact that in his second paper (1926) he was able to obtain an increase in tone in an involuntary muscle in a sodium chloride solution. In the case of the melanophores the contraction was just as marked in 0.1 M NaCl as in the isotonic Ringer's solution, although the recovery from ultra-violet contraction only took place in the latter. It seems probable, therefore, that calcium ions are not necessary for the production of contraction in the melanophore.

CONCLUSIONS

The experiments have not given any very definite knowledge as to the mechanism of contraction in melanophores. However, further evidence has been brought forward in favor of Spaeth's hypothesis that the contraction of a melanophore is similar to the contraction of smooth muscle. The partial contraction of the melanophores in response to ultra-violet

radiation seems to be similar to the increase in tonus found in smooth muscles after radiation which has been reported by Azuma and Hill.

The absence of any evidence of an isoelectric point in the response of the melanophores to ultra-violet radiation, in solutions of different pH, makes it seem unlikely that the contraction is due to a reversible colloidal aggregation.

Very satisfactory evidence has been found to show that a small amount of ultra-violet radiation produces an increase in irritability, but that larger doses produce decrease in irritability and eventually death.

A dose of 1.5 lithopone units gives the maximum increase in irritability. All doses up to 4 lithopone units cause some rise of irritability. Doses greater than this result in loss of irritability and 27.5 lithopone units is the lethal dose.

Experiments on the permeability of the melanophores to chemical agents after a maximum stimulating dose of radiation show that the resulting increase in irritability is not due to an increase in permeability.

The energy of ultra-violet radiation that will cause a maximum increase in irritability in an isolated pigment cell has been measured, and found to be the same whether the ultra-violet radiation is used alone or in combination with visible and infra-red light.

These results show that only short radiations with ultra-violet light are stimulating to isolated cells and that longer radiations, even though sublethal, produce a depressing effect. This emphasizes the fact that accurate dosage is extremely important in ultra-violet therapy. Where radiation is used to stimulate physiological processes, as in the healing of wounds, care should be taken to find the exact dose that gives the maximum stimulus for the process in question, as radiation in excess of this amount may produce the opposite effect.

The author wishes to express her appreciation to Dr. Janet H. Clark for her active interest and stimulating supervision during the work on this problem.

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THE EFFECT OF PITUITRIN ADMINISTRATION UPON THE DISTRIBUTION OF INJECTED FLUID

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When appreciable quantities of fluid are administered to an animal in excess of its immediate needs, readjustments take place whereby this extra fluid is rather promptly eliminated. It has been known for some time that the administration of extracts of the posterior lobe of the pituitary gland are able to modify the rate of this readjustment. The literature is entirely too voluminous to permit a review of even the investigations that have to bear directly upon the subject of this report. For this purpose the reader's attention is called to the recent papers by Geiling (1926), Craig (1925), Molitor and Pick (1924), Fromherz (1923) and Smith and McClosky (1924). A perusal of the literature convinces one of the fact that extracts can be prepared from the posterior lobe of the pituitary gland which when administered are capable of influencing the course of this regulation. It is a controversial point as to the nature of their action and the possible mechanisms involved. Many of the conflicting results are due, no doubt, to the great variance of experimental condition employed by the various investigators, as regards condition of the experimental animal, nature of and dose of extract employed, mode of administration and duration of the periods of observation. It is with the latter that this report is chiefly concerned. It appeared to us that most of the conflict resulted from a failure to distinguish between results due to effects of pituitrin per se and those resulting from readjustments occurring after its action had terminated. With this in mind we have studied the effect of pituitrin administration on the distribution of injected fluid between the blood, urine and tissues under experimental conditions which minimized the uncertainties of absorption and duration of action.

METHODS. Female dogs were given a standard diet and accustomed to the routine procedure. They were deprived of food and water for 20 hours previous to any experiment. Experiments were carried out on ten animals in which Ringer's solution was injected into the saphenous vein by means of a Woodyatt pump at the rate of 15 or 25 cc. per kilo body weight per hour for a period of two hours. At times from 0.05 to 0.08 cc. of commercial pituitrin (Parke, Davis & Co. or Eli Lilly & Co.) per kilo body

weight per hour was added to this solution. Experiments with Ringer's solution alone and Ringer's solution with added pituitrin were run on all animals. Ether was administered by the open cone method in several experiments in order to study the combined effects of pituitrin administration and anesthesia. The order of procedure was alternated in the experiments on different animals. Experiments were carried out on two animals in which a hypertonic solution of five times the saline content of normal Ringer's solution was injected. The hypertonic solution was injected at the rate of 15 cc. per kilo body weight for a period of one hour. The periods of observation included the period of injection and a post-injection period of three hours. Blood was drawn from the jugular vein before, just before the end of the injection and at the close of the post-injection periods. The drawing of frequent samples from the marginal ear vein was found unsatisfactory. Blood volume changes were calculated from the averages of hemoglobin and plasma protein changes. Hemoglobin was determined by Palmer's method. Plasma protein changes were determined by the refractometric method. A constant correction was made for the non-protein constituents of the plasma. Cell volume was estimated by means of a Van Allen hematocrit. For comparative purposes 7 per cent of the body weight was considered the average preliminary blood volume. The urine was collected by continuous catheterization. The small volume of urine that the animals would have passed without fluid injection has been neglected in the calculations. The percentage of injected fluid taken up by the tissues was calculated from the difference between the amount injected and that found to be in the blood and urine. Frequent observations were made of heart and respiratory frequency and rectal temperature.

Results. The administration of pituitrin to unanesthetized animals (see table 2) did not affect the amount the urine excreted during the period of injection of an isotonic or hypertonic Ringer's solution. However when the injection was conducted under ether anesthesia, the average values showed a greater urine output. This diuretic effect was more marked in experiments with the hypertonic solution. The animals when injected with Ringer's and pituitrin excreted almost twice as much urine in the 3 hour post-injection period as when the same animals were injected with Ringer's alone. This apparent diuretic effect of pituitrin in the post-injection period was not observed when the injections were made under ether anesthesia. When the averages for the entire period of observation were compared, it was noted that the administration of pituitrin increased the urine output and that ether anesthesia was antagonistic to this effect.

Table 1 shows the effect of pituitrin administration upon the concentration of hemoglobin and plasma proteins in animals which were injected with isotonic and hypertonic Ringer's solution. When one cal-

culates the amount of blood dilution (table 2) present at the end of the injection period, it may be seen that the animals receiving pituitrin and iso-

TABLE 1*

The effect of injections upon hemoglobin, plasma protein and body temperature

NATURE OF INJECTION	HEMOGLOBIN PERCENTAGE†		PLASMA PROTEIN PERCENTAGE			TEMPERATURE CHANGE DURING INJECTION
	End of injection	End of 3 hour post-injection	Before injection	End of injection	End of 3 hour post-injection	
						°F.
Ringer's	86.9	98.5	7.46	6.18	6.64	−0.26
Ringer's and pituitrin	75.0	93.2	7.38	5.43	7.25	−1.34
Ringer's, pituitrin and (ether anesthesia)	95.3	95.0	6.97	6.28	6.68	−2.80
Ringer's (nephrectomy)	90.5	97.5	7.27	6.18	6.93	
Ringer's, pituitrin (nephrectomy)	74.0	82.5	7.02	5.03	5.67	
Hypertonic Ringer's	79.0	99.5	6.83	5.82	6.85	+0.15
Hypertonic Ringer's, pituitrin	79.5	92.5	6.12	4.99	6.38	−0.30
Hypertonic Ringer's, pituitrin and (ether anesthesia)	115.0	104.5	6.30	5.96	6.23	

* Table based on average values.

† Hemoglobin before injection equals 100 per cent.

TABLE 2*

Distribution of fluid

NATURE OF INJECTION	PERCENTAGE OF FLUID				
	Excreted during injection	Found in blood stream	Calculated to enter tissue	Excreted in 3 hour post-injection	Excreted in both periods
Ringer's	20.9	26.7	52.4	28.7	49.6
Ringer's and pituitrin	21.0	53.4	25.8	51.9	72.9
Ringer's, pituitrin and (ether anesthesia)	26.9	11.3	61.6	30.9	57.8
Ringer's (nephrectomy)	0.0	25.5	74.5	0.0	0.0
Ringer's, pituitrin and (nephrectomy)	0.0	65.4	34.6	0.0	0.0
Hypertonic Ringer's	46.6	86.4	−32.9	124.0	170.5
Hypertonic Ringer's and pituitrin	52.1	85.2	−37.3	161.9	214.3
Hypertonic Ringer's, pituitrin and (ether anesthesia)	95.6	−19.6	24.0	102.1	197.7

* Table based on average values.

tonic Ringer's solution retained twice as much fluid in the blood stream as the same animals when injected with Ringer's solution alone. Approxi-

mately the same magnitude of effect was observed in experiments on two nephrectomized animals. This effect of pituitrin was not observed in the experiments with the hypertonic solution. Ether anesthesia was found to be antagonistic to this action of pituitrin. The calculated amount of fluid that must have been taken up by the tissues indicated that pituitrin administration permitted only about one-half as much fluid to be taken up by the tissues at the end of the injection period as in experiments with Ringer's alone and that this effect was counteracted by ether anesthesia.

No consistent change in rectal temperature was noted in animals receiving Ringer's solution alone. However when pituitrin was added to this solution a definite fall was usually found to have occurred at the end of injection periods. The animals receiving Ringer's solution showed either a slight increase or no change in pulse rate but when pituitrin was added an appreciable slowing was always observed.

Discussion. It is to be expected that, if pituitrin by any mode of administration causes either an increase or decrease in the rate of excretion of extra fluid, this effect, sooner or later, will be compensated for by a decreased or increased rate of elimination. When endeavoring to ascertain the action of pituitrin per se one must consider the results obtained during the period in which it is known to be available. The duration of its action is extremely uncertain when subcutaneous, intramuscular or intermittent intravenous modes of administration are employed. One is certain that during the period of continuous intravenous injection, pituitrin is available. We do not know with certainty how long its action persists in the post-injection period. However, its behavior under other conditions would suggest a rather transitory action.

In our experiments on unanesthetized animals the effect of pituitrin administration was to decrease the rate of removal of injected saline solution from the blood stream. This confirms the findings of Lamson and others (1923). The administration of pituitrin did not affect the amount of fluid removed by the kidney during the period of injection. When judged by this criterion alone its action was neither diuretic nor anti-diuretic. However, when one notes that the experiments in which pituitrin was administered showed a much greater blood dilution than the controls, a greater urine output in the former might be expected. In this action its effect should be regarded as anti-diuretic. If one considers only the total amount of urine eliminated in the combined injection and post-injection periods, the conclusions would be that pituitrin exerted a diuretic influence. Our records show that this extra urine output occurred only in the post-injection period and not during the time that pituitrin was known to be available. It occurred during the time that a greatly diluted blood was being restored to its original volume. It is our belief that this increased urine output represents for the most part the elimination of the extra fluid found in the

blood stream at the end of the period of injection and is an after readjustment to the conditions set up by pituitrin rather than an action of pituitrin per se. Without doubt the inability to recognize and properly evaluate this factor is responsible in a large measure for the many apparent inconsistent results obtained on the effect of pituitrin administration on urine output. The most apparent action of pituitrin in the experiments on the unanesthetized animal was that of greatly decreasing the rate of fluid removal from the blood stream and that this decreased rate of removal was due to the failure of the tissues to take up the usual amount of fluid.

We realize the possible inadequacy of the methods employed to accurately measure relative changes in blood volume as has been pointed out by Lamson and Nagayama (1920). The values obtained from changes in the concentration of hemoglobin and plasma proteins, although only considered as approximate, were of sufficient magnitude and constancy to show that the animals receiving pituitrin exhibited a much greater increase in blood volume than the controls with Ringer's solution alone. This confirms the findings of Underhill and Park (1923), Mackersie (1924) and others. This effect to pituitrin on blood dilution was not observed in the experiments with hypertonic Ringer's solution. However, in the experiments with the latter solution considerable quantities of water were obtained from the tissues as a vehicle for elimination and maintenance of isotonicity.

When the injections of Ringer's solution and pituitrin were made under ether anesthesia quite different results were obtained. The influence of anesthesia caused on the average a slightly greater urine output during the injection period. This was more marked when hypertonic solutions were injected. The extra urine output in the post-injection period was absent. The usual blood dilution was not noted and the quantity of fluid taken up by the tissues much greater. On the whole the result of ether anesthesia was antagonistic to many of the usual effects of pituitrin observed in the unanesthetized animal.

The methods employed in our experiments did not permit tissue analysis and determination of such factors as capillary area, pressure and permeability. A discussion of these factors and other possible mechanisms of pituitrin action would be a repetition of what has already been presented in several excellent reviews, and quite beyond the scope of this paper.

SUMMARY

1. Pituitrin when added to Ringer's solution and administered by the continuous intravenous method to unanesthetized dogs exerted no effect on the volume output of urine during the period of injection (usually a two-hour period). It caused a much greater blood dilution and a decreased rate of removal of the injected fluid by the tissues.

2. Its administration was followed by a greater urine output in the three-

hour post-injection period. We believe that this increased urine output represents primarily the elimination of the extra fluid from the blood stream, during the time of abating pituitrin action, rather than a diuretic action of pituitrin per se.

3. When injections of pituitrin and Ringer's solution were made in dogs under ether anesthesia only a slight dilution of the blood was noted. The volume of urine excreted during the injection period was, on the average, somewhat increased. The quantity of fluid calculated to have been taken up by tissues exceeded that found in experiments on the unanesthetized animal with either Ringer's solution alone or Ringer's and pituitrin.

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THE REGULATION OF CIRCULATION

VII. The Effects of Anoxemia of Mild Degree on the Cardiac Output of Unnarcotized Dogs

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Previous investigations (Harrison and Blalock, 1927) have shown that severe anoxemia of short duration causes an increase in the minute cardiac output. This work, as well as the observation that anemia if rapidly produced leads to increased cardiac output (Blalock and Harrison, 1927), suggests that the minute cardiac output may be inversely proportional to the tissue oxygen tension. If such a ratio exists, breathing pure oxygen should diminish the output. But breathing oxygen has no effect on the output of the heart, and therefore inverse proportionality between oxygen tension and minute blood flow only exists when the tension of oxygen is below a certain level. This threshold must lie between 95 per cent (normal) and 70 per cent arterial saturation, because Harrison and Blalock observed that the minute cardiac output was always increased when the arterial blood was less than 70 per cent saturated with oxygen.

The purpose of the present communication concerning the effects of mild anoxemia is to establish—between narrow limits—this anoxemic threshold. Throughout this paper "severe" anoxemia denotes arterial saturation less than 70 per cent, and "mild" anoxemia denotes arterial saturation between 90 and 70 per cent. This entirely arbitrary separation has been chosen for the sake of convenience and brevity.

Method. All experiments reported were carried out on healthy, normal, unnarcotized dogs which had been trained to the methods. A few additional experiments were done on animals which had received barbital and many observations were made on morphinized dogs. The results in the narcotized group were qualitatively similar to those in the trained dogs, but the quantitative findings were considerably less uniform. As this study is primarily concerned with quantitative relationships between two physiological variables we have felt that the findings in the narcotized animals were much less trustworthy for the purpose. Consequently, only the observations on trained unnarcotized dogs are reported.

The minute cardiac output was determined according to the Fick method, the technique being that described by Harrison and Leonard (1926). The rubber mask designed by Blalock (1926) was used for measurements of oxygen consumption.

In one group of experiments the cardiac output while breathing air was compared with that while breathing oxygen. In another series of observations, anoxemia was induced by rebreathing in a closed circuit, the CO_2 being absorbed. The minute cardiac output was determined before, during, and in some instances, after anoxemia. The rebreathing method has certain objections. The degree of anoxemia is constantly increasing; the percentage saturation of the arterial and venous bloods is steadily diminishing; and the oxygen consumption may change from minute to minute as anoxemia becomes more severe. Therefore, by this method there are two variables—a time factor, and a factor concerned with the degree of oxygen lack.

Because of these possible fallacies in the rebreathing method it was felt that our observations should be repeated using a constant gas mixture. A few experiments were carried out in this manner. After the control study the animal was attached to a Tissot spirometer containing a gas mixture composed of oxygen—7 to 12 per cent—and nitrogen—the remainder. (Because our nitrogen was impure the inspired air contained 1 to 2 per cent CO_2. However, Marshall (1926) has demonstrated that breathing such concentrations of CO_2 does not affect the cardiac output.) The expired air was collected in another Tissot spirometer. Two or more five-minute periods were run until the inspired mixture had all been breathed. Toward the end of each period simultaneous arterial and venous blood samples were obtained from the left and right ventricles. The oxygen consumption was measured by analysis of the inspired and expired air. In this way the degree of anoxemia was kept constant during the experiment, and the time factor constituted the only variable.

Results. A. *The effects of breathing oxygen.* Slight average diminutions in pulse rate and oxygen consumption were observed. These changes were not significant and are to be attributed to the fact that the determinations while air was breathed were done first and hence the animals had been on the table longer and were more nearly "basal" when the determinations while breathing oxygen were carried out. Benedict and Higgins (1911) have shown that breathing oxygen does not change the oxygen consumption.

The minute cardiac output when oxygen was respired was in each of the nine experiments within 10 per cent of the minute cardiac output when air was being breathed. The average change in minute output was −1 per cent.

B. *The effects of anoxemia.* The results obtained when the rebreathing

method was used are shown in table 1 and the findings with the "constant mixture method" are presented in table 2.

As the data obtained by the two methods are in fairly close agreement they are discussed together.

The *pulse rate* was not strikingly changed. During anoxemia a slight increase—10 to 20 beats—was noted in approximately one half of the experiments. In other instances the pulse rate was unchanged or diminished. These results are in accord with those found by Harrison and Blalock with more severe degrees of anoxemia.

The *oxygen consumption* was usually slightly increased although in several instances diminished oxygen consumption was found. Except for

TABLE 1

The effects of mild anoxemia on the cardiac output (rebreathing method)

EXPERIMENT NUMBER	WEIGHT OF DOG	BEFORE ANOXEMIA				DURING ANOXEMIA				PERCENTAGE CHANGE	
		Pulse rate	Oxygen consumption per minute	Arterial saturation	Cardiac output per minute	Pulse rate	Oxygen consumption per minute	Arterial saturation	Cardiac output per minute	Oxygen consumption	Minute cardiac output
	kgm.		*cc.*	*per cent*	*cc.*		*cc.*	*per cent*	*cc.*		
N_{11}	11.4	82	131	94	2340	78	132	67	4050	+1	+72
		96	136	94	2560	One hour after anoxemia					
N_{13}	13.6	132	173	94	2780	128	189	71	3360	+9	+21
N_{15}	8.9	140	91	93	2260	150	102	86	1540	+12	−33
N_{19}	16.0	128	136	95	2710	144	146	68	2890	+8	+7
N_{20}	10.5	84	87	93	1610	86	89	89	2360	+2	+46
N_{22}	12.0	120	115	95	2310	120	130	88	2360	+13	+2
N_{23}	9.0	84	89	89	2790	90	91	71	3140	+2	+12
A_4	13.4	98	127	95	2590	80	142	72	2370	+12	−8
A_5	11.4		108	94	1710		112	84	1720	+4	+1

two experiments, in which a decrease of approximately 25 per cent occurred, the changes in oxygen consumption were not of sufficient magnitude to be significant.

The *minute ventilation* was increased 50 to 100 per cent during anoxemia. This finding is in accord with Gesell's (1925) conceptions of respiratory control.

The respiratory quotient during the control periods was between 0.71 and 0.86. During anoxemia the R. Q. was always increased—0.80 and 1.57 being the lowest and highest values, respectively. The increased carbon dioxide elimination is to be attributed to over ventilation and is to be considered as the cause of the slight alkalosis which has been repeatly demonstrated to occur in the initial stages of anoxemia.

The *concentration of oxygen in the inspired air* (in the "constant mixture" experiments) varied between 11.38 per cent and 7.85 per cent. These values corresponded to 75 and 50 per cent arterial saturation, respectively. With a given mixture the arterial oxygen content remained remarkably

TABLE 2

The effects of anoxemia on the cardiac output ("constant mixture" method)

EXPERIMENT NUMBER	BEFORE ANOXEMIA						DURING ANOXEMIA								PERCENTAGE CHANGE	
	Pulse rate	Oxygen consumption per minute	Minute ventilation	Respiratory quotient	Arterial saturation	Minute cardiac output	Duration of anoxemia	Pulse rate	Oxygen consumption per minute	Minute ventilation	Respiratory quotient	Oxygen in inspired air	Arterial saturation	Minute cardiac output	Oxygen consumption	Minute cardiac output
		cc.	liters		per cent	cc.	minutes		cc.	liters		per cent	per cent	cc.		
A_{56}	80	96			95	2420	4	100	91	6.9	1.57	7.85	50	3280	−5	+35
							7.5	116	94	6.9	1.37		50	3820	−2	+58
	72	102			95	2020										
A_{58}	80	117	2.6	0.71	95	2040	4	75	86	3.9	1.01	10.73	75	1820	−27	−11
							11.5	88	89	3.9	0.92		75	1860	−24	−9
	104	112			95	1830										
A_{59}	88	57			95	1150	6.5	112	53	3.4	0.83	11.38	72	1330	−6	+15
							14.5	112	51	3.4	0.93		72	1200	−10	+5
	116	57	2.4	0.78	95	1270	21.5	118	59	3.7	0.80		73	1250	+4	+9
A_{61}	84	60	2.4	0.78	95	1430										
	88	75			95	1430	12	150	63	5.9	0.92	7.86	58	1780	−8	+25
							18	180	52	6.0	1.03		58	1770	−20	+24
	152	61			95	1430										
A_{62}	108	134	5.5	0.86	95	3270										
	156	134			95	3240	8	120	151	11.9	0.95	7.91	59	4990	+13	+53
							12	128	151	11.1	0.90		59	4610	+13	+42
	150	129			95	3270										
A_{63}	124	79	3.9	0.80	95	2020	8	136	100	7.7	0.85	8.26	59	2950	+25	+46
							13	142	85	7.2	0.85		60	2210	+7	+9
	160				95	1340										

constant. This indicated that in experiments of such short duration there was no compensatory increase in the hemoglobin content.

The *percentage arterial saturation* varied between 89 and 50. (Oxygen capacities were determined in some experiments. In other instances the

control arterial blood was assumed to be 95 per cent saturated and the oxygen capacity was calculated on this basis.)

The *minute cardiac output* was always increased when the arterial blood was less than 70 per cent saturated; with higher arterial saturation the changes were less uniform.

In four experiments the percentage saturation was between 80 and 90. In two of these the minute cardiac output was not changed. In one, there was a great increase (46 per cent) and in the remaining experiment a marked diminution (33 per cent) in cardiac output. The average change for these experiments was plus 4 per cent, which cannot be considered as significant.

In two observations the arterial blood was between 75 and 80 per cent saturated. The average change in cardiac output was −10 per cent. This cannot be regarded as important because of the small number of experiments.

Six determinations were made when the arterial saturation was between 70 and 75 per cent. In one of these a slight diminution (8 per cent) in minute output was observed. In the remainder the minute output was increased (5 to 25 per cent). The average change was plus 9 per cent. It is believed that this result is significant in indicating that 75 per cent arterial saturation is approximately the "anoxemic threshold" for normal dogs.

In three experiments the arterial blood was between 60 and 70 per cent saturated. The average change in minute cardiac output was plus 29 per cent.

Seven observations were made when the arterial saturation was between 50 and 60 per cent. The smallest increase in cardiac output was 24 per cent and the greatest increase was 58 per cent. The average change was + 40 per cent.

The *time factor* appeared to be unimportant. In one experiment the cardiac output was plus 35 per cent after four minutes and plus 58 per cent after 7.5 minutes of anoxemia. In another observation the minute output was plus 46 after eight minutes and plus 9 after 13 minutes. In the remaining experiments the cardiac output remained constant during the fifteen or twenty minutes of anoxemia. It is possible that anoxemia of longer duration might raise the threshold but of this we have no proof.

A typical experiment, showing the effects of anoxemia of increasing severity is plotted in figure 1.

Discussion. Our experiments indicate that the minute cardiac output of normal dogs is always increased when the arterial blood is less than 75 per cent saturated. There are some reports of similar findings by other investigators. Thus, Sands and DeGraff (1925), calculating cardiac output from the pulse pressure and pulse rate, stated that "increased systolic dis-

charge, an increased rate of the heart and a reduced peripheral resistance all combine during anoxemia to increase the minute flow of blood through the body." Dreyer (1926), using the cardiometer, found increased cardiac output in decerebrate cats at extreme degrees (50 to 80 per cent unsaturation) of anoxemia. Kisch (1926), by over-ventilation in curarized dogs caused the arterial saturation to diminish to 70 per cent. Under these con-

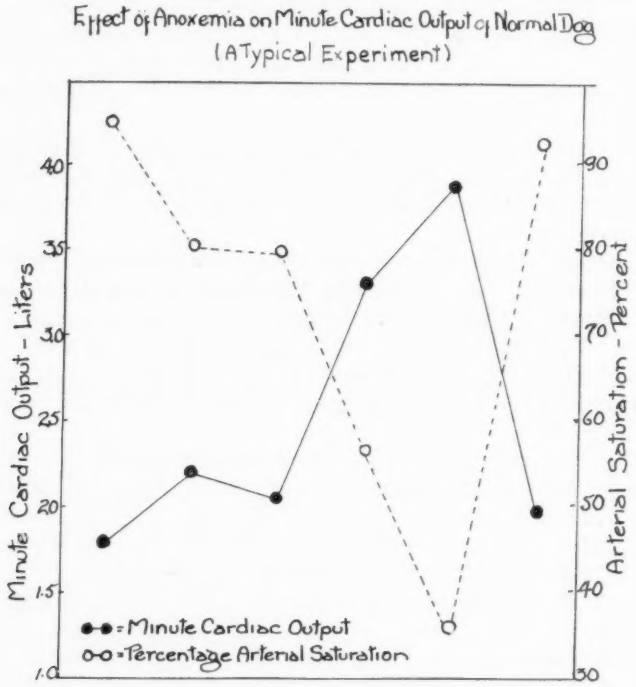

Fig. 1. This chart represents a rebreathing experiment. As the arterial saturation approached 80 per cent a slight rise in cardiac output was noted. At 55 per cent saturation the cardiac output was nearly double the control value and the increase was still greater at 35 per cent. When the anoxemia was terminated the percentage saturation and the cardiac output returned to approximately normal values. In this chart, time relationships are neglected.

ditions the cardiac output was increased. Doi (1922), using urethanized cats and the Fick method, found no change in minute output with arterial saturation as low as 81 per cent. His results cannot be considered as contrary to ours as we found no change at arterial saturations greater than 75 per cent.

However, a number of observers have reported different results.

Jarisch and Wastl (1926) observed cardiac dilatation and increased systolic volume with diminished output when the arterial blood was less than 60 per cent saturated. Their animals had had urethane and had been subjected to vagotomy and thoracotomy. Their results can only be interpreted as meaning that an animal which has been subjected to severe trauma cannot respond normally to a stimulus.

Gremels and Starling (1926) observed that anoxemia caused at first no change and then a diminution in the systemic output of the heart. They used the heart lung preparation and their dogs had initial cardiac outputs of approximately 300 cc. As this is only one-fourth to one-fifth of the cardiac output of normal dogs of average size, one is forced to conclude that the operative procedure and the low initial outputs were responsible for the failure of their animals to react normally to anoxemia. It would be interesting to know whether oxygen lack could cause increased cardiac output in the heart lung preparation if the initial output were maintained at a normal level.

Schneider and Truesdell (1923, 1924) observed no change or a diminution in the blood flow through the hand during anoxemia. They attempted to determine the cardiac output by the method of Barcroft, Roughton and Shoji (1921) and observed no change or a decrease in minute output and in stroke output. However, they admitted that the method used was unsatisfactory at low oxygen tensions. Caution must be observed in interpreting the relationship between the blood flow through an extremity and that through the entire body. Thus Bronk and Gesell (1926) observed that low oxygen tension caused diminished femoral and increased carotid flow of blood.

This brief review may be summarized by saying that the results reported by other workers have been inconstant. All previous observations, except those on man, have been on anesthetized animals. Nearly all the work has involved thoracic operations. The studies on man have been concerned with the blood flow of one part of the body only, or have necessitated the use of indirect, unreliable methods.

On the contrary our results were obtained on normal unanesthetized dogs, which were subjected to no operative manipulation. The method—the direct Fick procedure—has been repeatedly shown to be accurate. In contrast to the conflicting reports of others, our reports have been strikingly uniform. This leads to the conclusion that studies on the circulation in order to be applicable with certainty to the normal animal must be made on normal animals. This point of view has been expressed by others—notably by Marshall (1926).

The data presented in this paper, and in the previous work of Harrison and Blalock, seem to indicate that the cardiac output is always increased when the arterial blood is less than 70 per cent saturated, and usually not changed at arterial saturation greater than 80 per cent. The "anox-

emic threshold" therefore, seems to be approximately 75 per cent for normal dogs. All of the six dogs which have been used in these studies appear to have approximately the same threshold, although it is possible that some animals are slightly more sensitive than others to oxygen lack.

Whether the "anoxemic threshold" remains constant under all conditions cannot be stated. It is possible that conditions of stress, such as muscular exercise, may render the tissues more sensitive and raise the threshold. Possibly acids—by shifting the dissociation curve—adrenalin or muscle metabolites may have such an effect. It is likely that increased metabolic rate may raise the threshold.

Harrison and Blalock (1926) observed increased cardiac output in dogs with pneumonia. The degree of increase was correlated with the degree of anoxemia—even when the arterial blood was more than 80 per cent saturated. This observation suggests that the "anoxemic threshold" is higher in pneumonic dogs than in normal dogs.

In the final evaluation of these factors whereby the circulation responds to increased demand it is important to know: 1, the mechanism by means of which oxygen lack causes increased cardiac output; and 2, whether the sensitiveness of this mechanism to deficient oxygenation remains constant or is increased during physiologic stress. The answer to the first of these propositions is presented in the following paper; the second question remains unanswered.

SUMMARY

The cardiac output of normal, unanesthetized dogs has been studied by the Fick method. Breathing oxygen does not change the cardiac output. When the arterial blood is less than 70 per cent saturated with oxygen the minute cardiac output is increased. At arterial saturations greater than 80 per cent the minute output is usually not changed. The first increase in cardiac output usually comes when the arterial blood is approximately 75 per cent saturated. Seventy-five per cent saturation has therefore been designated as the "anoxemic threshold."

Anoxemia usually causes a slight increase in pulse rate and great increases in minute ventilation and respiratory quotient. The oxygen consumption is usually not significantly altered until the degree of anoxemia becomes extreme.

When the rebreathing method is used the results are similar to those obtained when the degree of anoxemia is kept constant with a low oxygen mixture.

It is a pleasure to express our appreciation to Dr. E. K. Marshall for valuable suggestions in regard to this work.

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THE REGULATION OF CIRCULATION

VIII. The Relative Importance of Nervous, Endocrine and Vascular Regulation in the Response of the Cardiac Output to Anoxemia

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Previous investigations (Harrison and Blalock, 1927) have shown that the minute cardiac output of normal unnarcotized dogs and of morphinized dogs is increased by severe anoxemia. Further studies (Harrison, Wilson, Neighbors and Pilcher, 1927) have demonstrated that mild anoxemia has a less striking effect, but that the minute output of the heart is usually increased when the arterial blood is less than 75 per cent saturated with oxygen. The reasons for the apparent discrepancy between these findings and those of other workers were discussed in the previous publications and need not be mentioned here. Evidence was presented for the view that the tissue oxygen tension is an important factor—although probably not the sole factor—in the organic control of the amount of circulation.

The present investigation has been concerned with an attempt to find the mechanism whereby anoxemia causes increased cardiac output. *A priori* there are several possibilities.

1. *Nervous regulation—A medullary circulatory center.* At various times since the discovery of the medullary center regulating the minute volume of respiration, the suggestion has been made that an analogous center regulating the minute volume of circulation existed in the medulla. Such a mechanism might function directly through nerve impulses to the heart or indirectly through nerve impulses to the organs of internal secretion.

2. *Endocrine regulation.* The work of Odaira (1925) showing that the internal secretions of the thyroid, adrenals, hypophysis and pancreas have effects on minute output raised the question as to the importance of the endocrine glands in the normal regulation of circulation. Since Cannon, Linton and Linton (1924) have shown that muscle metabolites may increase the rate of the completely denervated heart by increasing the secretion of adrenalin; since asphyxia causes increased adrenal secretions

(Cannon and Corrasco-Formiguera, 1922; Hartman, McCordack and Loder, 1923; Kodama, 1924); and since adrenalin increases cardiac output (Odaira, 1925; Lyon and Sands, 1925; Pilcher, Wilson and Harrison, 1927) it seemed that the adrenal glands might be responsible for the increased minute cardiac output during anoxemia. Cannon and Griffith (1922) showed that the liver contained a substance which could cause a change in the rate of the denervated heart. It seemed possible, therefore, that the liver might play a rôle in the circulatory response to anoxemia.

3. *Autonomous vascular regulation.* Krogh (1924) observed that anoxemia caused dilatation of the capillaries of the frog's tongue. Anrep and Bulatao (1925) using the heart-lung preparation, reported experiments indicating that the cardiac output is a function of the coronary flow, whereas Hilton and Eichholtz (1925) found that anoxemia caused increased coronary flow. There is thus direct evidence that anoxemia has an effect on the heart and on the vascular periphery. It seemed possible,therefore, that the regulation of circulation might be primarily an autonomous function of the vascular system.

In this study attempts have been made to discover whether the circulatory response to anoxemia is dependent on the central nervous system, the endocrine glands or the vascular system *per se*. The results may be summarized by saying that the experiments indicate the importance of intrinsic vascular control and the relative unimportance of extrinsic (non-vascular) factors.

Methods. Most of the experiments were carried out on morphinized dogs. A few observations were made on trained unnarcotized dogs. These are indicated by asterisks in the tables. Anoxemia was produced by rebreathing in a closed circuit, the carbon dioxide being absorbed. The cardiac output was determined according to the Fick principle which can readily be applied to trained unnarcotized animals (Marshall, 1926). For further details of our technique in determining cardiac output the reader is referred to our previous reports.

Cardiac denervation—partial or complete—was produced by several different procedures: 1, *vagotomy.* 2, *Complete thoracic sympathectomy,* according to the method of Cannon, Lewis and Britton (1926). By this technique the stellate ganglia and the entire thoracic sympathetic trunks (down to the ninth dorsal nerves) are removed. Cannon, Lewis and Britton have shown that removal of the stellate ganglia alone does not entirely isolate the heart from the sympathetic trunk. The operation was performed under ether and the animal allowed two hours to recover from the anesthetic before the blood flow studies were begun. 3, *Complete denervation—vagotomy plus sympathectomy.* Cannon, Lewis and Britton cut the vagal cardiac branches in the chest of their cats and saved the animals. We have not been able to keep our dogs alive for more than a

few days after the operation as they died of pneumonia. Therefore, our experiments had to be carried out within a few hours. Under these conditions it has been more convenient to cut the vagi high in the neck and save the added difficulty of cutting the vagal thoracic branches at the time of the operation for sympathetic denervation.

4. *Shutting off the blood supply to the head by ligating the carotid arteries and the subclavian arteries proximal to the origin of the vertebrals.* For reasons which are discussed later this method of denervation has not been as satisfactory as the more complete and direct methods mentioned above.

5. *The administration of atropine.* This procedure gives only vagal denervation but was used because the experiments could be carried out on trained animals requiring no operative procedure and hence served as a partial check on the operative experiments in which morphine was usually used.

Studies were made of the effects of the various denervation procedures on the minute cardiac output and of the effects of anoxemia on the minute output in the denervated animals.

Adrenalectomy. The operations were done under ether anesthesia. In most of the experiments both glands were removed at one operation, and the observations on the circulation were begun one to two hours later. In several instances each gland was removed at a separate operation as recommended by Stewart and Rogoff (1926). In a few experiments a loose silk ligature was inserted under the lumbo-adrenal veins proximal to the glands and the animal allowed several hours to recover from the operation. Studies were then made before, and at intervals after twisting the ligatures and thereby occluding the veins. It was possible in this way to study the effects of shutting the glands out of circulation without having the results confused by the anesthetic and the operation. Observations were made of the effects of adrenalectomy on minute cardiac output and of the effects of anoxemia on the minute output of the adrenalectomized animals.

The other endocrine organs. It is believed that the other organs of internal secretion are less important than the adrenals in so far as any immediate primary action on the minute cardiac output is concerned. Consequently, these were "ruled out" in groups. The pituitary, parathyroid and thyroid glands were excluded from the circulation by occluding the carotid and subclavian arteries at their sites of origin from the aorta. This procedure is almost equivalent to decapitation and thus causes cardiac denervation in so far as a cortical or medullary "center" is concerned. (Cerebral anemia causes a rise in blood pressure and hence any surprising results from the effects of anoxemia in such animals would have to be interpreted in the light of blood pressure changes, which were not recorded. However, as the results of anoxemia after ligating these vessels—see below—were not

different from the effects in normal animals it is not believed that this objection is valid.)

The *pancreas, liver, adrenals* and *gonads* were shut off from the circulation by ligating the inferior vena cava immediately above the diaphragm. Observations on the effects of anoxemia were carried out one or two hours after the operation. It is unlikely that within this period sufficient collateral circulation could develop to invalidate the results.

In regard to the various surgical procedures and the interpretation of the findings after them, one point should be emphasized. Any anesthetic or operative manipulation renders a dog abnormal. It is not to be expected that a sick animal can react to a normal stimulus to as great a degree as a normal animal. Consequently, we feel that negative results, i.e., instances in which anoxemia failed to cause an increased minute cardiac output—after these various procedures are of much less significance than positive results. This point is discussed more fully below.

RESULTS. A. *The effects of denervation:* 1. *Vagotomy.* The findings are shown in figure 1.

The usual increase in pulse rate was observed when the sympathetics were intact. When the sympathetics had been previously cut, vagotomy caused only a slight change in pulse rate. This finding indicates the presence of a sympathetic tonus. In one of the six experiments a rise in oxygen consumption occurred. In the remaining experiments the oxygen consumption was practically unaffected. The minute cardiac output was decreased 15 per cent in one experiment and increased 11 per cent in another. Otherwise, the changes in minute cardiac output were 10 per cent or less. The average changes after vagotomy were plus 5 per cent and minus 2 per cent for oxygen consumption and minute cardiac output, respectively.

These findings are similar to the observations of Toppan and Torrey (1926) who reported that cutting the vagi had no effect on the minute cardiac output of morphinized dogs.

2. *Atropine.* The results are presented in figure 1.

After the drug the pulse rates of the trained dogs were usually approximately doubled and the rates of the morphinized dogs were nearly quadrupled. Nevertheless, the minute cardiac outputs were unaffected by atropine, for in seven of nine observations after the drug the values were within 10 per cent of the control level. The average change in minute cardiac output after atropine was plus 4 per cent. The average change in oxygen consumption was plus 2 per cent.

These observations are in accord with those of E. K. Marshall (1926) who found that atropine had no effect on the minute cardiac output of his trained dogs. His findings and ours indicate that the minute cardiac output is relatively independent of pulse rate. The stroke output is very variable—the minute output surprisingly constant.

3. *Sympathectomy.* The relation of the cardiac sympathetic nerves to the cardiac output has been made the subject of a separate study by Blalock and will be reported in detail at a later date. The experiments

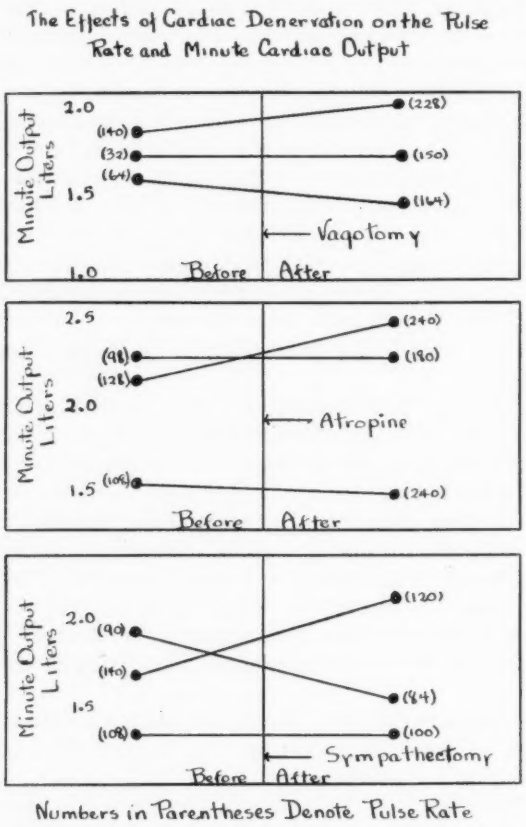

Fig 1. Time relationships are neglected. Vagotomy and the administration of atropine produced increased pulse rate but had practically no effect on the minute cardiac output, the changes being in each instance less than 10 per cent—which is the maximum limit of error of the method. Sympathectomy produced slight diminution of the pulse rate in each instance. The cardiac output per minute was unchanged once, increased by 15 per cent once and diminished by 15 per cent once. As the control determinations were made before the operation and the observations after sympathectomy were made two hours after the operation it is not believed that changes of such small degree can be considered significant.

charted in figure 1 represent typical results. As would be expected after such an extensive operation there is often some change in the minute output,

but these changes are not constant and can probably be attributed to the operative procedure *per se*. In the three experiments charted (fig. 1) the cardiac output once decreased approximately 15 per cent, once increased the same amount and once was unaltered. It is noteworthy that the pulse is usually somewhat slower after sympathectomy. This observation is in accord with that concerning the effect of vagotomy on the pulse rate

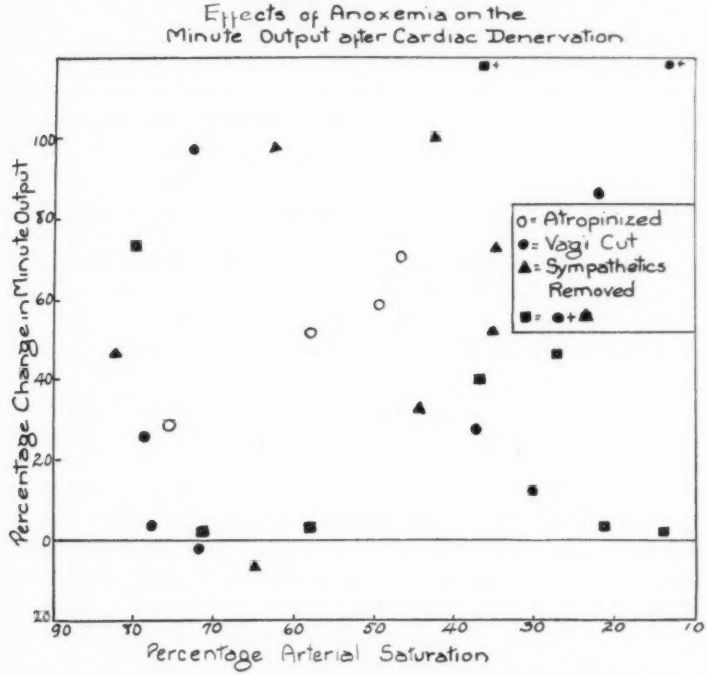

Fig. 2. Anoxemia produced increased cardiac output after cardiac denervation. The effects were most constant in the atropine experiments (hollow circles) which were done on trained animals. The effects were least constant in the completely denervated dogs (squares) which had been subjected to the greatest amount of trauma. Except for the three symbols in the lower right hand corner, this chart is not materially different from similar ones constructed from experiments on normal dogs. The conclusion is drawn that the cardiac nerves are not responsible for the increased minute output during anoxemia.

of previously sympathectomized dogs. Both findings indicate that there is normally some degree of sympathetic "tone."

B. *The effects of anoxemia after cardiac denervation:* 1. *Anoxemia in vagotomized dogs* (fig. 2). A slight increase in pulse rate, usually about 10 beats per minute, was the rule. "Mild" anoxemia—we use the term

arbitrarily to denote those instances in which the percentage arterial saturation is between 70 and 90—caused no change in minute output in two other instances. "Severe" anoxemia (arterial blood less than 70 per cent saturated with oxygen) was induced in four experiments and caused an increased minute output in each instance. The smallest increase was 12 per cent, the greatest 127 per cent. These results correspond to those found in previous observations on animals with intact vagi and indicate that these nerves play no important rôle in the circulatory response to anoxemia.

2. *Anoxemia in sympathectomized dogs* (fig. 2). In six of seven observations the minute output was strikingly increased. In the remaining instance a slight diminution—7 per cent—was found. The arterial saturation was 65 per cent in this experiment, and therefore, not much below the "anoxemic threshold" (Harrison, Wilson, Neighbors and Pilcher) for normal dogs. The usual change in pulse rate was an increase of 10 to 30 beats per minute. From these experiments the conclusion is drawn that the cardiac sympathetic nerves play no rôle or a relatively unimportant one in the circulatory response to anoxemia.

3. *Anoxemia in atropinized dogs.* Most of the other groups of observations were made on morphinized animals. Consequently it was believed that studies on trained unnarcotized dogs denervated by atropine would afford an additional check on the accuracy of the findings in the morphinized animals. As can be seen from figure 2, the minute cardiac output of the atropinized dogs was strikingly increased during anoxemia. The smallest increase was 29 per cent and the largest increase was 65 per cent. The more constant findings in the group are to be attributed to the fact that trained animals were used and no operative procedure was necessary.

In studying the circulation of dogs under various conditions we have been constantly impressed by the uniformity of results in normal animals as contrasted to the variations often observed in anesthetized dogs after operations. It seems likely that some of the present conceptions of circulatory physiology and circulatory pharmacology must be revised. Thus digitalis, which usually causes increased minute cardiac output when plethysmographic methods are used (De Heer, 1912; Wiggers, 1927) has the opposite effect in normal dogs (Harrison and Leonard, 1926); strychnine which increases the minute cardiac output of normal dogs (Wilson, Pilcher and Harrison, 1927) has practically no effect on animals in shock (Blalock, 1927). Caffeine, which increases minute cardiac output of dogs in a state of shock (Blalock, 1927) usually causes a diminution in normal animals (Pilcher, Wilson and Harrison, 1927); the minute cardiac output of dogs under ether anesthesia increases with increase in pulse rate (Toppan and Torrey, 1926) whereas no such relationship holds in normal animals (Marshall, 1926).

4. *Anoxemia in completely denervated dogs.* After sympathectomy and vagotomy anoxemia caused increased minute cardiac output in four instances. In four other observations no change—plus 1 to plus 3 per cent—was found.

The lack of uniformity in the results can probably be attributed to the operations and anesthetics incident to the denervation procedure. The greater the operative procedure the less capable may the animal become of responding to a normal stimulus. The marked increase in minute cardiac output observed in one-half the experiments indicates that the circulatory response to anoxemia is not primarily dependent on the cardiac nerves and would seem to exclude the possibility of any medullary circulatory center reacting to changes in oxygen tension and controlling the circulatory minute volume through nerves to the heart.

The effect of anoxemia in animals which had been subjected to the various denervation procedures is portrayed in figure 2. Inspection of this chart brings out two chief points; *a*, The cardiac output is nearly always increased, and *b*, the uniformity of the circulatory response is in direct proportion to the amount of manipulation the animals have suffered. The circles indicating atropinized animals fall almost in a straight line, suggesting a quantitative relationship between the degree of anoxemia and the percentage change in minute output. The squares, denoting completely denervated animals, show the least uniformity—probably because these animals had been subjected to more extensive operative procedures. The symbols denoting sympathectomized dogs and those indicating vagotomized animals are intermediate in their degrees of variability. This was to be expected as these animals had been subjected to more trauma than the atropinized group and less trauma than the completely denervated dogs.

C. *The effects of anoxemia after exclusion of the various endocrine glands from the circulation.* 1. *Anoxemia after ligation of the carotid and subclavian arteries.* Occlusion of these vessels at their sites of origin must cause either total ischemia or a very marked diminution in the amount of blood passing through the thyroid, parathyroid and pituitary glands, consequently the circulatory response to anoxemia, if normally dependent on hormones from these organs, should be absent or much diminished. Such was not the case. In table 1 four observations during anoxemia are shown. In each instance the minute cardiac output was much greater than that during the control period. The average increase was approximately 35 per cent. Moderate elevations in pulse rate and variable changes in oxygen consumption were found. These are comparable to what one finds in normal animals from similar degrees of anoxemia. As would be expected the degree of increase in cardiac output was slightly less than that which usually occurs in normal dogs. This is probably to be attributed to the operative procedures.

It is noteworthy that carotid and subclavian ligations constitute a denervation procedure. Our animals continued to breathe spontaneously for a time after occlusion of the vessels and hence there must have been some medullary circulation—probably from anastomosis with the spinal arteries. (Spontaneous respiration after occluding these vessels was also noted by Rogoff and Coombs (1923).) However, the amount of circulation to the brain must have been very much diminished and therefore the finding of increased minute output during anoxemia in these animals is further evidence of the unimportance of the central nervous system in the regulation of the minute cardiac output.

2. *Anoxemia after ligation of the aorta and inferior vena cava.* When these vessels are ligated in the thorax the minute cardiac output undergoes

TABLE 1

The effects of anoxemia with the subclavian and carotid arteries occluded

EXPERIMENT NUMBER	WEIGHT OF DOG	CONTROL PERIOD BEFORE ANOXEMIA				DURING ANOXEMIA				PERCENTAGE CHANGE	
		Pulse rate per minute	Oxygen consumption per minute	Arterial saturation	Minute cardiac output	Pulse rate per minute	Oxygen consumption per minute	Arterial saturation	Minute cardiac output	Oxygen consumption	Minute cardiac output
	kgm.		*cc.*	*per cent*	*cc.*		*cc.*	*per cent*	*cc.*		
A_{10}	10.5	104	56	95	1150	148	66	54	1640	+20	+43
						160	75	48	1530	+36	+33
A_{12}	8.2	78	79	95	1110	82	88	46	1490	+11	+34
						86	42	20	1430	−46	+29
			88	90	1050	Control after anoxemia					

striking, progressive diminution. All the phenomena of shock except lowered blood pressure develop (Blalock and Johnson, 1927). Within one to four hours after the operation the animal dies of shock. When the cardiac output is in process of decreasing, a striking rise during anoxemia is scarcely to be expected. Under these conditions a slight rise should be significant. The results are shown in table 2.

Four determinations of minute cardiac output were made during anoxemia and after ligation of the aorta and inferior vena cava, above the diaphragm. A marked increase (114 per cent) occurred in one experiment. A moderate increase (27 per cent) and a slight increase (10 per cent) were also observed. A slight diminution (9 per cent) was found in the fourth instance. The slight changes can not be regarded as significant because they occurred when the arterial saturation was slightly above the usual

"anoxemic threshold" of normal dogs. "Severe" anoxemia caused an increased minute output. These findings which suggest that the organs below the diaphragm are not responsible for the circulatory response to anoxemia, seem to exclude the influence of hormones from the pancreas, liver, gonads and adrenals in causing the increased minute output. However, as the adrenals have been shown to be stimulated by asphyxia, it was felt that further and more direct experiments were necessary before concluding that they were not essential for the normal increase in circulation produced by low oxygen tension.

3. *Anoxemia in adrenalectomized dogs.* The effects of adrenalectomy *per se* on the minute cardiac output are shown in figure 3. One of us (Blalock) has made several similar observations of a progressive diminu-

TABLE 2

The effects of anoxemia after ligation of the aorta inferior vena cava

EXPERIMENT NUMBER	WEIGHT OF DOG	CONTROL PERIOD BEFORE ANOXEMIA					DURING ANOXEMIA				PERCENTAGE CHANGE	
		Pulse rate per minute	Oxygen consumption per minute	Arterial saturation	Minute cardiac output	Output per minute per kgm.	Pulse rate per minute	Oxygen consumption per minute	Arterial saturation	Minute cardiac output	Oxygen consumption	Minute cardiac output
	kgm.		*cc.*	*per cent*	*cc.*			*cc.*	*per cent*	*cc.*		
A_{46}	14.6	156	58.9	98	470	32	152	64.3	81	520	+9	+10
A_{50}	10	138	37.8	95	360	36	145	39.6	83	330	+5	−9
							142	41.4	39	770	+10	+114
A_{54}	10	14.4	47.0	94	400	40	156	28.2	45	510	−40	+27

tion in minute output after removal of these glands. The normal minute cardiac output of healthy, trained, unnarcotized dogs weighing five to twenty kilograms, is not less than 100 cc. and not more than 200 cc. per kilo of body weight (Marshall, 1926; Harrison and Blalock, 1926). In anemic dogs the minute output may be more than this (Blalock and Harrison, 1927). Small dogs usually have a somewhat greater cardiac output per kilo than do large dogs. The minute output per kilo in the adrenalectomized dogs was, with one exception, always below the average value for normal dogs, which is approximately 150 cc. per kilo. In two-thirds of the experiments the minute output after adrenalectomy was below the minimum normal values—100 cc. per kilo. The longer the time after adrenalectomy the lower the minute output became.

The importance of diminished cardiac output in "adrenal shock" does

not seem to be generally recognized. Blalock (1927) has demonstrated that cardiac output is a much more delicate and accurate indicator of shock following hemorrhage than is mean blood pressure. It seems likely that many of the chemical changes described in adrenalectomized animals are simply indications of shock and are due to diminished blood flow. Swingle (1926) reported increased phosphorus and non-protein nitrogen in the blood after bilateral epinephrectomy in cats. Elevated non-protein

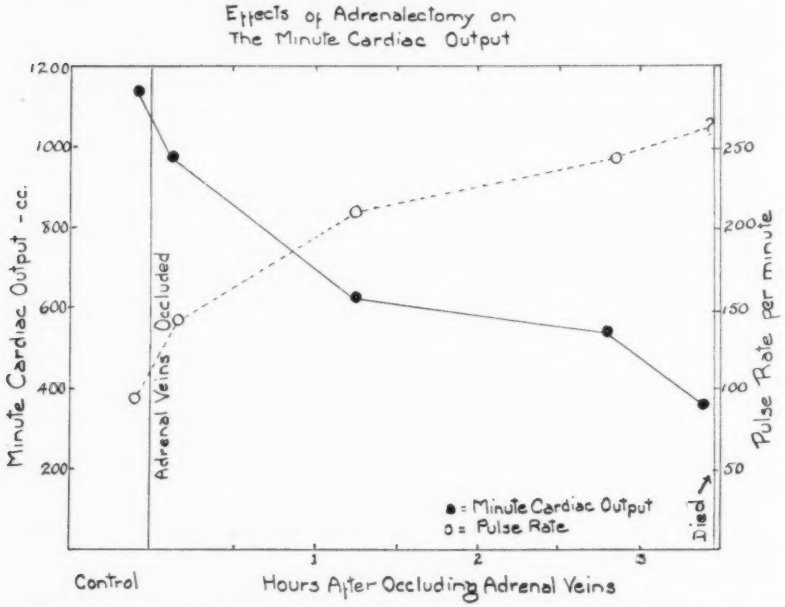

Fig. 3. This experiment is typical of the effects of adrenalectomy on minute cardiac output. At the operation the glands were not removed but loose silk ligatures were inserted around the lumbo-adrenal veins. Two hours later the animal was in good condition. The control cardiac output was determined and the veins were then occluded by twisting the ligatures. The progressive diminution in cardiac output and the increasing pulse rate are shown. Just before death the pulse rate was so rapid that it could not be counted accurately.

nitrogen is found in shock (Aub and Wu, 1920) and increased blood phosphorus content is seen in conditions where the kidneys are severely damaged (Meyers, 1924). Possibly the increased concentration of these substances in the blood of adrenalectomized animals is not a specific adrenal effect but a "shock effect" secondary to diminished blood flow through the kidneys. Swingle and Eisermann (1926) found diminished serum bicarbonate, diminished carbon dioxide tension and increased hydrogen ion

concentration in their adrenalectomized cats. These effects may be produced by anoxemia (Koehler, Brunquist and Loevenhart, 1925) and shock causes marked tissue anoxemia dependent on diminished blood flow (Blalock, 1927).

Corey (1927) observed that forcing fluids prolonged the survival time of adrenalectomized cats. He stated: "It is not clear why forcing fluids should prolong the life of bilaterally epinephrectomized cats. The idea which most readily comes to mind in this connection is that the forcing of fluids and the consequent diuresis flushes some toxic substance out of the organism." Blalock (1927) has shown that the administration of saline solution causes marked increase of the cardiac output of animals in shock, and hence the improvement following the forcing of fluids in adrenalectomized animals may be readily explained without assuming the existence of a toxin.

The effect of adrenalectomy on the minute cardiac output constitutes, therefore, suggestive evidence that the essential immediate effect of adrenalectomy consists of circulatory shock, and that other immediate effects are secondary to the shock level of blood flow.

In this connection, it is of some interest to point out that we have not been able to keep our animals alive for more than a few hours after a one-stage bilateral adrenalectomy and for not more than two days after removal of the second gland in animals which had unilateral adrenalectomy. However, Stewart and Rogoff (1926), who have performed the operation hundreds of times reported an average survival period of more than one week for their dogs when the adrenal glands were removed in two operations. Their experience with the operation and careful after-care allows the animals to survive the initial shock period, which is almost certainly dependent on the removal of the adrenal medulla, and which has been uniformly fatal to our animals. We do not believe that chemical changes *occurring in the first few hours or days* after removal of the glands can be interpreted otherwise than as secondary effects of shock. Stewart and Rogoff (1926) reached the same conclusions.

The degree of shock must be considered as an important factor in evaluating the effects of anoxemia in the circulation of adrenalectomized dogs. In figure 4 the minute cardiac output during anoxemia is portrayed in three different groups of animals. In the group with normal minute cardiac outputs during the control period and in the group with mild shock anoxemia usually caused a moderate or marked increase in cardiac output. In those animals with severe shock, i.e., very marked diminution of the minute output during the control period, anoxemia usually caused a further diminution. The animals which responded to anoxemia by increased cardiac output could uniformly withstand longer rebreathing and lower oxygen tensions than could those animals which were unable to increase

their cardiac outputs. This finding is illustrative of the importance of increased blood flow as a compensatory mechanism during anoxemia.

Another point is of interest. The cardiac output per minute per kilo was much less in the animals which had had the aorta and vena cava ligated (table 2) than in the sickest adrenalectomized animals. None of the latter lived long enough to develop such low blood flows—30 to 40 cc. per kilo—as were observed in the "aorta and cava" group. And yet, those

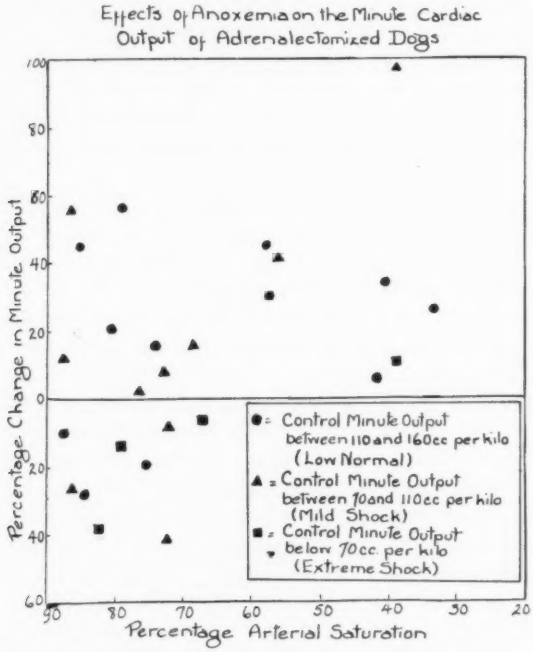

Fig. 4. In these experiments all the animals were in some stage of shock. The circles represent those with incipient shock—cardiac outputs at the lower limits of normal, and the triangles represent dogs in mild or moderate stages of shock—cardiac outputs below the lowest normal level but more than one-half of the average for normal dogs. Most of these animals responded to anoxemia with increased cardiac output. The squares represent dogs in states of very severe shock—cardiac outputs less than one-half of the average for normal dogs. These were unable to increase their cardiac outputs during anoxemia. When the chart was constructed, none of the symbols "fell" in the lower right hand corner in the space occupied by the legend. No animal which did not increase his cardiac output could withstand severe degrees of anoxemia. This indicates the compensatory nature of the circulatory response to low oxygen tension.

The conclusion drawn from this chart is that adrenalectomy does not abolish the normal circulatory response to anoxemia, but that severe shock, from any cause, may do so.

adrenalectomized animals which had very low cardiac outputs responded to anoxemia with a decrease, whereas the "aorta and cava" dogs with an even lower output responded normally to anoxemia. And the latter animals were also adrenalectomized so far as any functioning of these glands was concerned. This discrepancy is probably to be attributed to two facts. In the first place the blood pressure remains normal after ligating the aorta and vena cava (Blalock and Johnson) whereas the blood pressure is reduced in severe "adrenal shock." In the second place, the cardiac output was greater *per unit of living tissue* in the dogs with the vessels ligated. Everything below the diaphragm is practically dead tissue in such experiments and although the total blood flow is very low—one-fourth or one-fifth normal—the volume flow per unit of living tissue was probably not as low in these animals as in the adrenalectomized dog. It is probable that the heart was better supplied with blood in the dogs with "mechanical shock" than in those with "adrenal shock" and hence could respond more adequately to the stimulus of anoxemia.

Discussion. The data presented in this paper seem to demonstrate that the central nervous system and the endocrine glands can be excluded so far as any primary influence on the circulatory response to low oxygen tension is concerned. It appears likely that auto-vascular control plays the essential rôle. Attempts have been made to determine whether the heart or the vascular periphery is primarily responsible for the increased circulatory minute volume during anoxemia. Cross circulation experiments were attempted but these were unsuccessful.

Some evidence on this point is available, however. The observations of Krogh, who found capillary dilatation during anoxemia, has been mentioned. Hilton and Eichholtz found that anoxemia caused increased coronary flow, while Anrep and Bulatao believed that the cardiac output is a function of the coronary flow. Sands and De Graff (1925) found increased pulse rate, increased output per beat and diminished peripheral resistance during anoxemia. The experiments in the adrenalectomized animals with shock offer some evidence on this point. Many types of shock, of any significant degree, are accompanied by great diminution in minute cardiac output (Blalock, 1927). The venous oxygen content is very much diminished. Before arterial anoxemia was induced the adrenalectomized animals had some degree of tissue oxygen lack. Why did not this lead to increased cardiac output? Does arterial anoxemia exert different qualitative tissue effects from those produced by stagnant anoxemia? There seems to be an explanation for this apparent paradox, if we assume, as Cannon and Cattell (1922) suggested, that during the early stages of shock—i.e., after the minute cardiac output has decreased but before the mean blood pressure has changed significantly—the flow of blood through the peripheral organs is reduced but the volume flow through the heart is

maintained at a normal level. Under such conditions there would be lowered oxygen tension in the peripheral tissues but not in the heart, and this organ should be functionally capable of responding to a normal stimulus. Arterial anoxemia, which of course affects the heart no matter how efficient the coronary blood flow may be, would under such conditions cause increased cardiac output *provided the increased cardiac output during anoxemia is due to a primary cardiac effect and not to a peripheral vascular effect.* This is what occurred in the animals in mild shock (fig. 4).

On the other hand, in severe shock, when the cardiac output is still lower and the mean blood pressure markedly decreased the coronary flow is probably much impaired (Cannon and Cattell). Under these conditions it is not to be expected that the anoxemic stimulus acting on an impaired organ could be effective. In our animals in severe shock, anoxemia usually caused a decreased minute cardiac output (fig. 4).

The reaction to anoxemia of the dogs which had been subjected to ligation of the aorta and inferior vena cava is of interest in this connection. These animals had cardiac outputs which were only about 20 per cent of normal. But the mean blood pressure is normal in such animals until immediately before death (Blalock and Johnson). Normal mean blood pressure implies relatively normal coronary circulation. These animals were able to respond to anoxemia with an increased cardiac output.

It is very difficult to explain the reaction of the animals with incipient adrenal shock and of those with mechanical shock to anoxemia on the hypothesis that the circulatory response is primarily dependent on the peripheral vascular system, because in mild shock there is already a severe degree of oxygen lack in the peripheral tissues. This evidence, although indirect, is very suggestive of the *hypothesis that the tension of oxygen in the coronary blood—and hence in the heart muscle—is the most fundamental physiologic factor in the regulation of the circulatory minute volume.*

If this be true, certain of the present concepts of circulatory physiology must be revised. The degree of cardiac filling—the extent of diastole—is ordinarily regarded as a passive mechanism, dependent on the pressure of the great veins. If the heart can (quite independently of the peripheral vessels) increase its minute output 100 per cent or more without changing its rate more than 20 per cent—as occurs during anoxemia—then diastolic filling under these conditions must depend on the degree of diastolic relaxation and must be controlled by changes in the tonus of the heart muscle and not by venous pressure.

There is, therefore, reason to believe that anoxemia affects both the heart and the vascular periphery. The effect on the heart seems to be much more important but this point cannot be regarded as definitely established until further evidence is available.

The demonstration that anoxemia causes increased minute cardiac out-

put does not conclusively prove that the tissue oxygen tension is the normal factor controlling the response of the vascular system to such a physiological state as exercise which necessitates increased blood flow. It appears that certain other factors play a rôle. One of these is the adrenals. Although these glands are not directly concerned in the circulatory response to anoxemia they are probably important in the circulatory response to exercise. Cannon, Linton and Linton have demonstrated that muscle metabolites cause increased adrenal secretion. According to Stewart and Rogoff (1919) the adrenal glands of cats and dogs normally liberate 0.00015 to 0.00036 mgm. of adrenalin per minute per kilogram. Pilcher, Wilson and Harrison (1927) found that the intravenous injection of adrenalin in doses of 0.025 mgm. per kilo caused an increase of approximately 15 per cent in the minute cardiac output. Therefore, the normal rate of secretion of adrenalin would have to be increased one hundred times in order to produce within one minute a significant change in cardiac output. This appears unlikely.

On the other hand removal of the adrenal glands causes a marked diminution in cardiac output and consequent shock which is believed to be the underlying cause of the *early* adrenal death. (In animals which survive this period and live for several days as in those of Stewart and Rogoff death must be attributed to the loss of the cortical tissue.)

The adrenal medullary tissue must be regarded, therefore, as a possible factor in the circulatory response to exercise. Our present knowledge is not sufficient to decide whether adrenalin is more important or less important than tissue oxygen tension. In any event, our experiments show that the two factors act independently.

The data on cardiac denervation point to the conclusion that the nervous control of the minute volume of circulation is considerably less important than the chemical control. The various denervation procedures neither altered the normal minute cardiac output, nor the response to anoxemia.

In normal dogs low oxygen tension causes marked changes in minute output and slight changes in pulse rate; the cardiac nerves exert a marked influence over pulse rate and practically no influence over minute output, and the adrenal hormone causes changes of intermediate degree in both the pulse rate and the minute output. Further work is necessary before the relative importance of these three factors in the normal circulatory response to conditions of physiological stress can be evaluated.

SUMMARY. The minute cardiac output of dogs during anoxemia has been studied by the Fick method. Low oxygen tension which increases the minute cardiac output of normal dogs has the same effect after vagotomy, after sympathectomy, after atropine and after complete denervation—sympathectomy and vagotomy. These various denervation procedures, some of which cause great changes in pulse rate, are uniformly without significant effect on the minute cardiac output.

Adrenalectomy causes progressive diminution in cardiac output, and a consequent state of shock. The various chemical changes which occur in the blood immediately after adrenalectomy can possibly be regarded as secondary to shock and diminished blood flow.

Anoxemia causes increased minute cardiac output in adrenalectomized dogs provided the degree of shock be not extreme. In adrenalectomized dogs in a state of extreme shock anoxemia is very poorly tolerated; the cardiac output diminishes still further, and death occurs at degrees of anoxemia which are easily tolerated by normal animals.

Ligation of the thoracic aorta and thoracic inferior vena cava causes progressive diminution in minute cardiac output which, however, increases during anoxemia.

In animals which have been subjected to ligation of the carotid and subclavian arteries at their sites of origin, anoxemia causes increased cardiac output.

The circulatory response to anoxemia is more constant and of greater degree in normal dogs than in animals which have suffered operations. The more abnormal the animal and the more artificial the conditions the more inconstant and inadequate is the response to the normal stimulating action of oxygen deficiency on the circulation.

CONCLUSIONS

1. In the functional control of circulatory minute volume chemical regulation is more important than nervous regulation. The cardiac nerves which are important in the control of heart rate play no rôle in the control of the cardiac minute output. There is no medullary circulatory center regulating minute cardiac output.

2. Chemical regulation of the circulation is of at least two general types: *1*, by hormones, and *2*, through the blood gases. The hormonal regulation appears to be chiefly adrenal and affects both the pulse rate and minute cardiac output. Anoxemia—below a certain threshold—affects the minute output to a marked degree and has a relatively slight effect on pulse rate. The circulatory response to anoxemia is independent of the nervous system and the endocrine glands, being due to a direct vascular action. Evidence has been presented which indicates that the tension of oxygen in the heart muscle is possibly the most important factor in the control of the circulatory minute volume.

It is a pleasure to express our appreciation to Professor Walter E. Garrey for advice and helpful suggestions.

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EFFECT OF DIFFERENT AMOUNTS OF SEXUAL INDULGENCE IN THE ALBINO RAT

III. Food Consumption[1]

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In the first two papers of this series (Slonaker, 1927a, b) we discussed the effect of different amounts of sexual indulgence on growth and on activity. The plan and scope of the experiment were described in detail in the first paper. In this paper the food consumption as influenced by sexual indulgence will be considered.

Owing to the fact that the animals kept in stationary cages were paired it was impossible to get their individual food consumption. The results which follow were obtained from twenty females which were isolated in revolving cages. These animals were divided into four groups of five each and subjected to the following sexual conditions: group A were virgins; group B were light breeders which delivered but two or three litters during their life span; group C were medium breeders, and had as many litters as they could produce and nurse to the weaning age; group D consisted of heavy breeders, whose young were destroyed at birth and the mothers rebred as rapidly and as often as possible. All these animals were kept in the same room, in similar cages, fed more than they would eat daily of the same well-balanced synthetic food, the only variable factor in their environment being the amount of sexual indulgence permitted. Any differences in the results we attributed to this one variable factor.

The animals were put in their respective revolving cages and the experiment started at the average age of 72 days. The age of each of the groups averaged A, 63 days; B, 83 days; C, 78 days; and D, 70 days. The amount of food eaten was ascertained by weighing to the animals a greater amount than they would eat in the interval between feeding times and weighing back the amount uneaten. This was done once each twenty-four hours.

We have previously shown in a former group of rats (Slonaker, 1925) that both gestation and lactation cause an increase in the daily food con-

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and the Committee for Research on Sex Problems of the National Research Council.

sumption. Gestation caused but a slight increase which was almost uniform in amount during the period except the last day or two when there was a slight decrease to the time of delivery. Lactation caused a progressive increase in the daily food intake from birth up to the time of weaning the young. The amount of increase was in direct correlation with the number of young in the litter. After the young were weaned the daily food intake of the mother returned to normal in a few days. This indicates

TABLE 1

Giving the average daily activity and food consumption during periods of twenty days preceding gestation and the first twenty days of gestation and of lactation

GROUP	NON-PREGNANT				GESTATION				LACTATION			
	Number of cases	Average age	Average daily activity revolutions	Average daily food	Number of cases	Average age	Average daily activity revolutions	Average daily food	Number of cases	Average age	Average daily activity revolutions	Average daily food
				grams				*grams*				*grams*
A.....	5	262	17,295	18.8	0				0			
B.....	15	214	12,951	20.8	12	237	5,078	20.9	8	229	2,483	26.3
C.....	9	265	5,322	17	19	290	3,422	20.1	15	271	1,480	29.6
D.....	21	307	5,935	19.3	30	281	3,491	22.2	0			

TABLE 2

Giving the average total run, food, maximum body weight, number of litters and average size for each group of animals

GROUP	AVERAGE TOTAL MILES RUN	AVERAGE TOTAL FOOD	AVERAGE MAXIMUM WEIGHT	AVERAGE NUMBER LITTERS	AVERAGE NUMBER OF YOUNG	AVERAGE SIZE LITTER
		grams	*grams*			
A.....	5,989	14,748	246	0		
B.....	3,851	17,150	350	2.6	12.6	4.85
C.....	1,696	15,966	328	6.4	44.0	6.88
D.....	2,468	16,209	329	6.6	47.4	7.18

that the growth of the young before birth requires a much less expenditure of energy on the part of the mother than their post-natal growth.

We have verified these former results in this experiment. This is shown in table 1. In this table we have given the average daily activity and food consumption of each group of animals for twenty days prior to gestation, the first twenty days of gestation and the first twenty days of lactation. This table shows some variations in the different groups. The average daily food consumption for all groups of non-pregnant rats was 19 grams; for the first twenty days of pregnancy it was 21 grams; and for the first twenty days of lactation it was 28 grams.

Owing to the increased food consumption during gestation and lactation we would expect a greater average total food consumption in those groups which bore young than in the group of virgin animals. Table 2 and figure 1 show that this was the case. The order of the average total food intake, from the lowest to the greatest was group A, virgins, 14,748 grams; group C, medium breeders, 15,966; group D, heavy breeders, 16,209; and group B, light breeders, 17,150 grams. The fact that group D, which did not nurse their young, had a greater average than group C which did rear

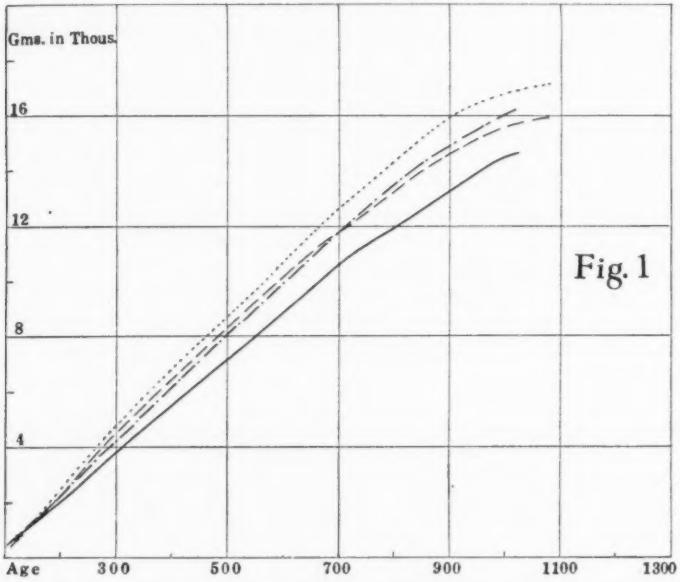

Fig. 1. Curves of total average food consumption for each of the four groups in revolving cages. Continuous line, group A, virgins; dotted line, group B, light breeders; dash line, group C, medium breeders; dot-dash line, group D, heavy breeders.

their young, which involved a greater expenditure of energy, seemed at first inconsistent. The average number of young delivered by each group was slightly greater in group D but not sufficient to over-balance the increased expenditure of energy in lactation by the C group. The average maximum body weights were almost identical. The cause for the differences in food intake in these groups seems to be influenced not only by bearing of young but also by the amount of energy expended in activity, lactation and growth. Group D carried 329 grams 772 miles farther than group C; and group B moved the heavier weight of 350 grams 2155 miles in excess of group C.

If group A, whose energy expenditure was restricted to growth, basal metabolism and activity, is used as a basis for computation to determine the amount of food required for each of these functions we will be able to arrive at the amount of energy expended in reproduction in the other groups. Thanks are due to my colleague, Dr. Victor E. Hall, for his great assistance in making these computations.

Mitchell and Carman (1916) in their study of the utilization of food energy in growing rats used a diet very similar in food value to the one used in our experiment. They found that about 15 per cent of the gross energy of the food was excreted in the feces and the urine thus leaving 85 per cent of metabolizable energy. In a former paper (Carman and Mitchell, 1926) they determined the body surface and the average basal heat production per square meter in a series of rats of different ages. Using these data they were able to calculate the energy expenditure in growth

TABLE 3

Giving the average total food consumed and the distribution of the energy output in each of the four groups

GROUP	AVERAGE MEAN WEIGHT	TOTAL MILES RUN	AVERAGE TOTAL FOOD		DISTRIBUTION OF ENERGY IN CALORIES			
			Grams	Calories	Loss in feces and urine	Growth and basic metabolism	Activity	Reproduction
A.....	212.5	5,989	14,748	58,107	8,716	21,654	27,737	0
B.....	293.4	3,851	17,150	67,560	10,140	28,261	24,261	4,509
C.....	275.7	1,696	15,966	62,940	9,441	25,479	10,200	17,820
D.....	277.2	2,468	16,209	63,870	9,581	25,498	14,920	13,871

and basal metabolism. These data were used in computing the energy expenditure in the different groups of our animals. These results are given in table 3.

The method of computation used in arriving at these results was to divide the average total life span of each group into thirteen periods and to determine the weight, body surface, and the growth and basal metabolism for the middle day of each of the periods. This was multiplied by the number of days in the period and the sum of these products gave the total calories used in growth and basal metabolism. The sum of this amount and the loss in feces and urine when deducted from the total amount eaten gave us, in group A, the energy expended in functional metabolism manifested in running. From this we were able to compute the energy used for each gram of body weight per mile of running activity. This amounted to 0.02181 calorie or an equivalent of 0.005536 gram of food for each gram of body weight. This was used in determining the energy expenditure used in functional activity in the other groups as given in table 3.

In the groups which bore young, B, C and D, the food intake was in excess of the energy expended in growth, functional and basal metabolism and the loss in the feces and urine. This excess of energy we have considered the amount necessary for the reproductive activities. Carpenter and Murlin (1911) have shown in the human that the basal metabolic rate of a pregnant woman is equal to that of the fetus plus that of the delivered woman. Harding (1925) in regard to metabolism during pregnancy says "pregnancy would thus appear to result in no alteration in the energy exchange beyond that produced by the growth within the maternal organism of a new mass of active protoplasmic tissue of a higher metabolic rate." We feel justified therefore in assuming that the increased food intake during pregnancy is due to reproduction and not to an incraesed basal metabolic rate of the mother. The greater increased intake of food during lactation

TABLE 4

Giving the energy intake and expenditure during the period of gestation and the first twenty-two days of lactation

GROUP	PERIOD OF GESTATION (22 DAYS)							FIRST 22 DAYS OF LACTATION						
	Food eaten		Energy used (calories)					Food eaten		Energy used (calories)				
	Grams	Calories	Loss	B.M.R.	Activity	Reproduction	Calories for 1 young	Grams	Calories	Loss	B.M.R.	Activity	Reproduction	Calories for 1 young
B	459.8	1812	272	554	582	404	83.21	578.6	2280	331	554	283	1112	326.9
C	442.2	1742	262	554	387	539	78.39	651.2	2566	385	554	169	1458	279.4
D	488.4	1924	290	615	433	586	81.65							

we consider due to the expensive turnover of energy of food into energy of milk.

Table 3 shows the expenditure of energy for reproduction in the order that would be expected. That is, group C, which reared almost the same number of litters and young as were born and destroyed at birth in group D (table 2), used the greatest amount of energy in reproduction and group B used the least.

If we estimate the energy intake for each young born we find that group B, which delivered the two or three litters early in life, ate an average of 104 grams for each young born (table 3). Group C averaged 90.7 grams and group D 20.7 grams. The average for the B and C groups, which nursed their young, was 97.35 grams per young born. These figures further substantiate the statement regarding an increased expenditure of energy during lactation. The greater intake of food per young born in group C than in group B may have been due to the reproductive activities occurring early in life in group B, while in group C they were extended throughout

the sexual life. But we have no data to substantiate this assumption at the present time. Another factor which may have been operative is the difference in the size of the litter (table 2). In group B the average size of the litter was 4.85 young while in group C it was 6.88 young.

Making use of the data at hand and using the results given in table 1 we were able to compute the average energy expenditure 1, for the period of gestation, and 2, for the first twenty-two days of lactation for each of the groups that bore young. From this we were able to determine the amount of energy required for the development of a single young during

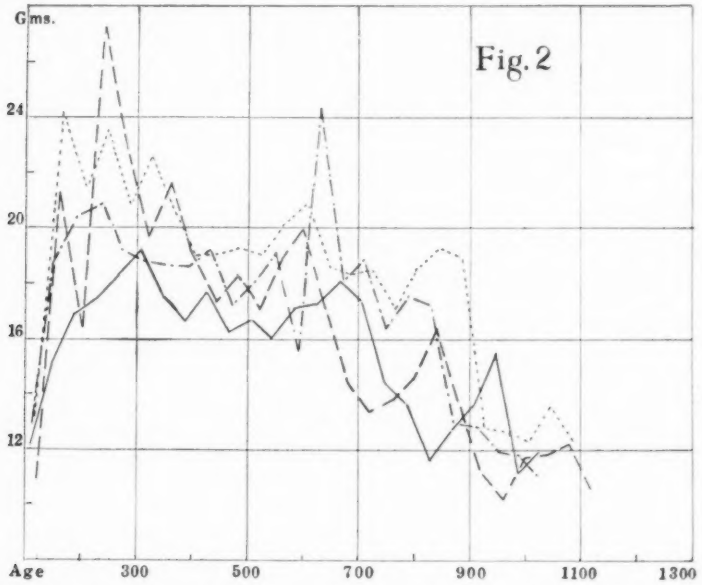

Fig. 2. Curves of average daily food consumption for each of the four groups in revolving cages. Averages of 40-day periods were used. Continuous line, group A, virgins; dotted line, group B, light breeders; dash line, group C, medium breeders; dot-dash line, group D, heavy breeders.

these two phases of reproduction. In computing the requirements during lactation allowance was made for the number of young that died and were not nursed. These results are given in table 4. This table shows that the energy expenditure per young born was greatest in the light breeding group, B, both in gestation and lactation, than in any of the other groups and that in the medium breeders, group C, it was the least. This may be at least partly explained by the difference in the average body weight of the mothers (table 2). This suggests that in group B more of the ingested energy was stored as fat or utilized in a greater body growth than in the other groups.

In figure 2 we have shown the average daily food consumption for each group during the life span. These curves represent averages of forty day intervals. Each group showed marked irregularities which may in part be due to the long fluctuations in activity previously described (Slonaker, 1926). In groups B, C and D the fluctuations in early life were largely due to reproduction. All groups showed a rapid increase in daily food intake to a maximum. This was followed by a general slight reduction to about 700 days of age after which there was a more rapid decrease during senility. The more gradual ascent of the curve of the virgins was due to lack of reproduction. It will be noted that the daily intake of food by the virgins was for the greater part below that of any of the breeding groups. Figure 2 also shows that the light breeders, group B, were equaled or surpassed in daily food consumption by any of the other groups only a few times during their entire life span.

SUMMARY

1. During the first twenty days of pregnancy the average daily food consumption was increased 2 grams and during the first twenty days of lactation 9 grams more than in non-pregnant rats.

2. Pre-natal growth of the young required a less expenditure of energy on the part of the mother than post-natal growth.

3. The average intake of food in virgin rats for activity was 0.005536 gram for each gram of body weight per mile run.

4. The amount of food required for the development of each young from the beginning of the gestation period to the weaning age was approximately 97.35 grams (groups B and C). For gestation alone it was 20.7 grams (group D).

5. The increased food consumption during gestation and lactation was greatly augmented by the pronounced reduction in voluntary activity.

6. Breeding females showed a more rapid increase in daily food consumption than virgin rats.

7. There was a direct correlation between the total food consumption and activity, growth and reproduction.

8. A greater expenditure of energy for reproduction was manifested in light breeders than in medium or in heavy breeders.

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THE GAS EXCHANGE OF ISOLATED MUSCLES DURING STIMULATION AND RECOVERY

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General reviews of the literature on the gas exchange of isolated muscles have been written by Verzar (1916) and, more recently, by Meyerhof (1925). A consideration of the experiments therein reported suggests the following three points: 1. There are no observations available showing continuous measurements of the oxygen consumption before, during, and after stimulation of muscles, small enough to permit rapid diffusion of gases. Adam (1921) gives indeed one incomplete experiment of this type. Thunberg (1906) gives continuous readings at 30-minute intervals after stimulation for 25 minutes. Meyerhof (1925) has published a diagram of the recovery oxygen consumption lasting for 24 hours. It seemed desirable, therefore, to study the time course of the excess oxygen consumption of small muscles stimulated for 5 to 10 seconds in a sensitive respirometer. 2. Previous studies of the respiratory quotient of frog muscle have been confined to observations on the total metabolism and no attempts have been made to determine the R. Q. of the *excess* metabolism due to activity. The usual procedure in studying the recovery oxygen consumption has been to fatigue a muscle more or less completely before putting it into the respirometer and then to determine the R. Q. for a certain period during recovery. In this way the relative time courses of the exchange of oxygen and carbon dioxide following stimulation have been overlooked and the first 15 minutes of recovery are lost. 3. There is a widespread belief, founded on not very extensive evidence, that the R. Q., at least of isolated muscle, is 1.0. This is indeed a very probable figure but it seemed that further determinations of this ratio by a different method would be of value.

EXPERIMENTAL. The apparatus for the experiments was the differential volumeter previously described (Fenn, 1927) similar to the one originally devised by Thunberg. It consists of 2 equal sized bottles (12–14 cc.) connected through suitable cocks by a fine capillary tube (1.94–2.66 c.mm. per cm.) carrying an index drop of kerosene. A small muscle (sartorius, semitendinosus or ileofibularis of the frog) is placed in one bottle in contact with sealed-in platinum electrodes. After a suitably constant basal rate of

movement of the index drop is obtained, the muscle is stimulated for 5 to 20 seconds with a tetanizing current (Harvard induction coil, one cell in primary, secondary at 10–12 cm. usually) and the further movements of the drop are then followed until the basal rate is resumed. In these small muscles the excess oxygen consumption is usually complete in 30 to 40 minutes. The animals used were mostly *R. pipiens*. The temperature was constant to <0.01°C. either at 22°C. or 12 to 15°C. according to the water bath used. The low temperature was satisfactorily and cheaply obtained by means of a cooling coil fed by continuous circulation from a tank of

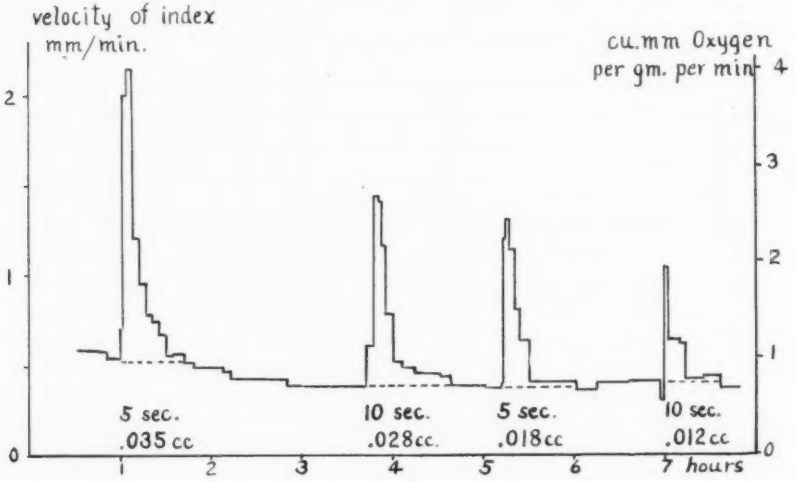

Fig. 1. Graphs showing the increased oxygen consumption of a semitendinosus muscle of the frog at 14.8°C. caused by brief tetanic stimulations of 5 to 10 seconds at 50 per second. Harvard induction coil, coil distance 10 cm., 1.5 volts in primary. The excess oxygen is given in cubic centimeter per gram of muscle. Weight of muscle, 194 mgm.

water kept in an adjoining cold room where the temperature was just above 0°C. Circulation in the coil was maintained by an air blast.

Results. 1. *Demonstration of an excess oxygen consumption due to stimulation.* The results of one representative experiment are shown in figure 1. Four periods of stimulation are plotted. At each stimulation the rate of movement of the index drop was immediately increased 4 to 5 times and then gradually returned to normal. The experiment makes a beautiful demonstration of the increased oxygen consumption of muscle due to stimulation and would seem to be an ideal experiment for teaching purposes. The result is, however, curiously difficult for students to obtain. In my experience frogs kept in the cold room for some days before pithing

are more likely to give success. Failure is due largely to injuries from inexpert handling of these small muscles, which seem to be surprisingly sensitive to slight stretches. Students have frequently reported to me that the muscle has actually contracted without an extra consumption of oxygen but a thoroughly satisfactory proof of this seemingly remote possibility is still lacking.

The data from another experiment similar to that of figure 1 are plotted in figure 2. In this case an ileofibularis muscle weighing only 48 mgm. was used. The frog had been kept for 4 days before killing in the cold room just above 0°C. Six stimulation periods were tried, two of them on the sec-

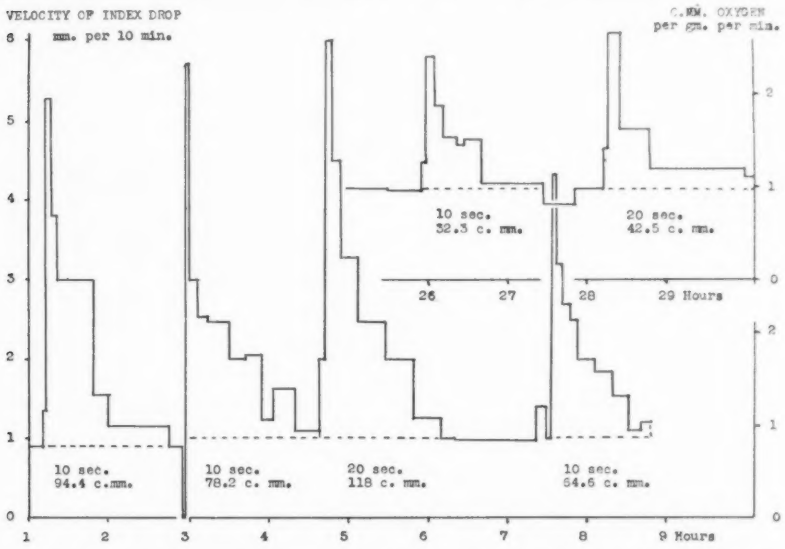

Fig. 2. Increased oxygen consumption of an ileofibularis muscle of the frog (weight 48 mgm.), caused by brief tetanic stimulations at intervals up to 28 hours after dissection. Frog kept 4 days in cold room before pithing.

ond day of the experiment 26 and 28 hours after dissection (at 15°C.) and in every case a pronounced increase in oxygen consumption was observed although there was a gradual diminution from one period to the next.

2. *Duration of stimulus.* Observations have been made, in all, during 45 stimulation periods similar to those in figures 1 and 2 and have yielded similar results. From some of these data a relation can be found between the duration of the stimulus and the excess oxygen consumption. Curves illustrating this point are plotted in figure 3 where ordinates represent the extra oxygen consumed per gram of muscle and abscissae represent the duration of the tetanus in seconds. In some experiments, of which that in

figure 1 is typical, the fatigue is so great that no comparisons are possible between stimuli of different durations. In other cases it is possible to alternate durations in such a way that the effects of fatigue are balanced out. The second, third and fourth stimulation periods of figure 2 are typical and are plotted as curve 2 in figure 3. The excess oxygen does not seem to be quite proportional to the duration of stimulation, there being relatively less used at the higher durations because of progressive accumulation of

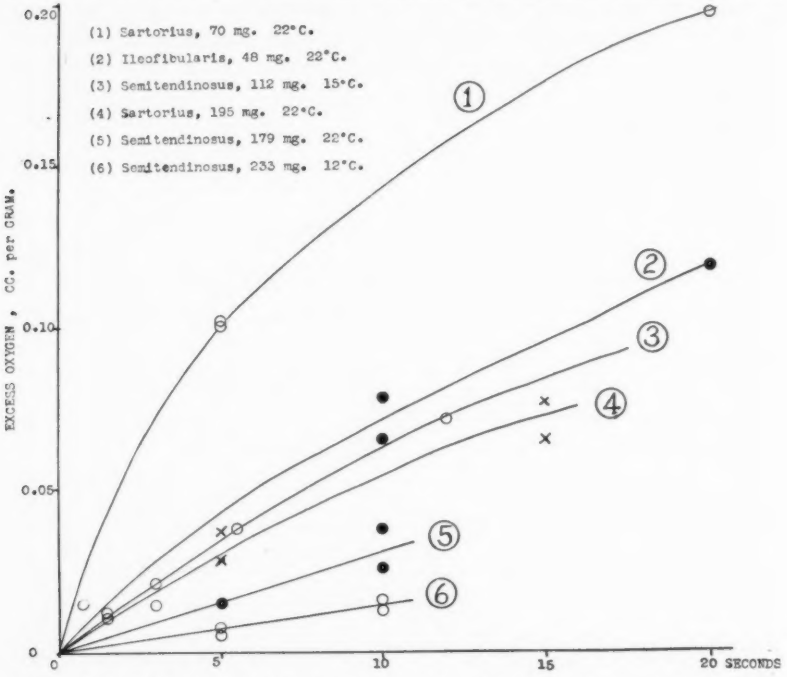

Fig. 3. Graphs showing the effect of varying durations of stimulation on the excess oxygen consumption of various frog muscles. The effect of temperature is obscured probably by the inevitable variations between individual muscles even when taken from the same frog.

lactic acid. It would seem to be true also from the data of figure 3 that small muscles of 70 and 48 mgm. (curves *1* and *2*) use more recovery oxygen for the same duration of stimulation than the larger muscles of 179 and 195 mgm. (curves *4* and *5*). Curve *6* might be low because of the lower temperature.

The average excess oxygen per 10 seconds stimulation in 45 stimulation periods was 0.0473 cc. per gram. The average lactic acid content of frog

muscles after 10 seconds tetanus according to Embden (1925) was 0.052 per cent in 8 experiments. Taking 0.016 per cent as the lactic acid content of resting muscles the increase due to stimulation was 0.036 per cent. The oxygen necessary to burn this lactic acid is 0.269 cc. per gram of muscle. Comparing this figure with mine the oxidative quotient turns out to be $\frac{0.269}{0.0473} = 5.7$. This compares well with many previous estimates of Meyerhof and of Hill, and shows that my values are of the right order of magnitude. The two sets of experiments are not sufficiently comparable to make this figure one of independent interest.

3. *The R. Q. of resting muscle.* The first apparently reliable determinations of the R. Q. of isolated muscles were those of Thunberg (1906) who reported an average of 1.06 in 8 experiments. Meyerhof (1919 and 1921) and Meyerhof and Meier (1924) have found almost identical values in frog muscles. Meyerhof and Himwich (1924) in the rat diaphragm find an average value of 0.95.

TABLE 1

Resting rates of gas exchange

cc. $\times 10^{-5}$ per gram per minute

O_2 AT 22°C.	O_2 AT 14.8°C.	$O_2 - CO_2$ AT 14.8°C. IN O_2	$O_2 - CO_2$ AT 14.8°C. IN 10 PER CENT CO_2
250	67	5	9.6
232	130	58	8.8
427	85	35	11.4
304	97	29	14.0
302	109	23	7.7
222	98	28	8.8
			9.9
284	127		6.9
176	77		13.5
			7.0
104			11.0
Average 255	99	30	9.8

The data of table 1 make possible another estimation of the R.Q. In this table are included the values observed for the resting oxygen consumption at 22° and at 14.8°C. and in the last two columns the rate of volume change in the absence of NaOH, i.e., the values of $O_2 - CO_2$ at 14.8°C. in an atmosphere of O_2 and 10 per cent CO_2 respectively. The rate of oxygen consumption at 22°C. is about $2\frac{1}{2}$ times as high as that at 14.8°C. As was observed by Cristina (1909) and doubtless by others, the index drop always moves toward the muscle chamber in the absence of NaOH. This does not

prove by itself, however, as has been supposed, that the R.Q. is less than 1.0 because the CO_2 tension is rising in the apparatus and therefore CO_2 is being retained.

In a separate series of experiments which will be published later, I have measured the solubility of CO_2 in muscle at different tensions, i.e., the CO_2 dissociation curve of muscle.[1] This curve is naturally much flatter at 10 per cent CO_2 than in pure O_2, which explains the fact that the drop moves toward the muscle chamber much less rapidly when the muscle is already saturated with 10 per cent CO_2. Thus $O_2 - CO_2$ is 30×10^{-5} cc. per gram per minute in O_2 but only 9.8×10^{-5} in 10 per cent CO_2 according to table 1. From solubility measurements it is found that 100 grams of muscle at 22°C. absorb 0.18 cc. CO_2 per mm. Hg rise in tension. If this value changes with temperature at the same rate as the solubility of CO_2 in water the figure at 14.8°C. would be about 0.22 cc.

Now if A is the observed CO_2 output per gram muscle per minute for a muscle of w grams in a bottle of v cc. capacity, then the rate of increase of the CO_2 tension is $\frac{Aw}{v} \times 760$ and the CO_2 absorbed will be $\frac{760 \times 0.22\ wA}{100v}$ or, assuming a muscle of 0.120 gram in a 12 cc. bottle, 0.0167 A. Therefore from these figures it is clear that in 10 per cent CO_2 the CO_2 retained will be only 1.7 per cent of the CO_2 liberated by the muscle. If it be assumed from table 1 that in 10 per cent CO_2 the oxygen consumption is 99×10^{-5} cc. then the CO_2 liberated is $99 - 9.8$ or 89.2×10^{-5} cc. The CO_2 retained is 1.7 per cent of this or 1.5×10^{-5} cc. and the R.Q. is $\frac{90.7}{99}$ or 0.92. Using this method, therefore, it may be concluded that the R.Q. is high but is uniformly less than 1.0 or about 0.92.

This result may be expressed in another way. By assuming an R.Q. of 1.0 the above formula may be solved for the solubility of CO_2 in the muscle which would make this R.Q. consistent with the experimental rates. It is found that if the observed CO_2 is $99 - 9.8$ and the R.Q. $= 1.0$, the solubility of CO_2 must be 1.45 instead of 0.22. An error of 650 per cent in this figure is impossible.

In defence of this conclusion it may be added *1*, that the maximum possible error in the determination of the slope of the CO_2 dissociation curve is only about 20 per cent; *2*, that the figures for $O_2 - CO_2$ in 10 per cent CO_2 mixtures are based upon readings taken 3 to 4 hours after inserting the mus-

[1] The full description of the method for measuring the CO_2 dissociation curve of muscle is not quite ready for publication. It depends, however, upon producing a sudden increase or decrease in the CO_2 tension in the muscle chamber and observing the movement of the index drop as the CO_2 is taken up. The absorption follows approximately an exponential curve and extrapolation to cover the 10 seconds involved in changing the gas involves no serious error.

cle in the respirometer so that ample time has been allowed for the establishment of the equilibrium diffusion gradients and a completely steady state must have been attained. After such a time the rate of movement of the index drop is only about 1 mm. in 30 minutes and is very constant. *3.* If values for the oxygen consumption even 100 per cent greater than 99 were assumed, the R.Q. would still be measureably less than 1.0. *4.* The only other possible interpretation of these results seems to be the possibility of a

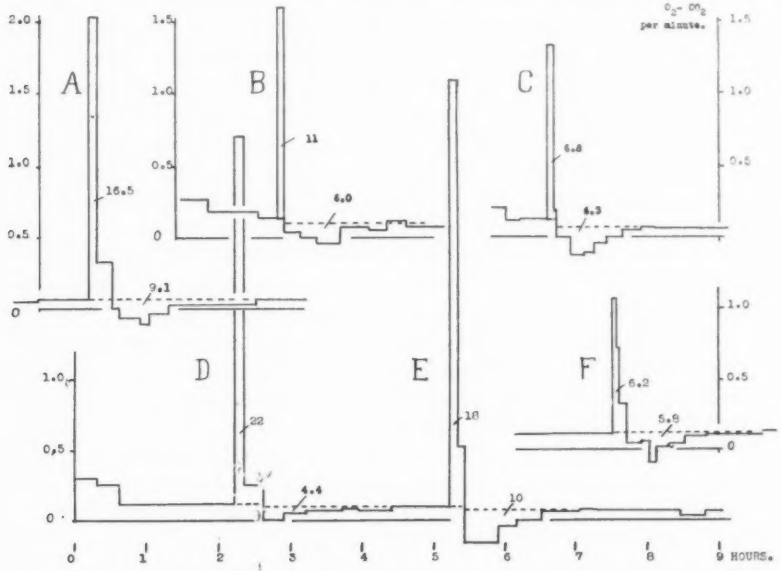

Fig. 4. Graphs showing the rate of movement of the index drop during 6 stimulation periods in the absence of NaOH. Ordinates represent values of $O_2 - CO_2$ in cubic millimeters per gram muscle per minute. Areas between the curves and the base lines (dotted) are given in cubic millimeters per gram of muscle. Where these two areas are nearly equal as in C and F, the R.Q. is very nearly 1.0. Calculations of the R.Q. and further data on these experiments are given in table 2. D and E were two successive stimulation periods on the same muscle.

constant production of ammonia or diminution of acidity in the muscle or incomplete oxidation.

4. *The respiratory quotient of the excess metabolism.* In previous studies of the gas exchange of isolated muscles no measurements have been made, so far as I am aware, of the respiratory quotient of the excess metabolism due to stimulation. I have attempted to study this matter with the respirometer described by stimulating the muscles in the absence of NaOH so that movements of the index drop indicated values of $O_2 - CO_2$. When this is

done in an atmosphere of *oxygen* there is an acceleration of movement of the index drop toward the muscle chamber indicating that the oxygen consumption is increased more than the CO_2 output. This is to be expected on account of the large retention of CO_2 at low tensions. The error involved in correcting such curves for the retention of CO_2 is so great that the resulting values for the R.Q. are not of much significance. In atmospheres of 10 to 20 per cent CO_2 in oxygen, however, very characteristic curves are obtained similar to some already described for nerves and these curves can be used to give a fairly dependable indication of the true R.Q. of the excess metabolism. The results of 6 such stimulation periods are plotted in figure 4. In every case the first result is a marked increase in the oxygen consumption. This is followed in 10 to 15 minutes by a relative increase in the carbon dioxide output so that the index drop may even reverse and move from the muscle chamber. This phase may persist for nearly two hours before the original basal rate is again regained. The base line which has been assumed in calculating the results has been drawn as a dotted line. It is frequently true that the base line falls slightly during the stimulation period. A similar fall of the base line may be found in unstimulated muscles observed over long intervals. In experiments in which the base line does not return to its original value after $2\frac{1}{2}$ to 3 hours after stimulation it is assumed that it never *will* return and the new base line is used in calculating the second phase of the experiment where the rate is less than the basal rate. Outstanding examples of this result are found in figure 4 *B* and *C*. This possibility of a shift of the base line together with the very prolonged recovery introduces a considerable error into the interpretation of the results. A slight shift in the value of the base line chosen makes a considerable difference in calculating the area of the rise or the fall in the graph. In other words, the method involves the calculation of the position in the capillary where the index should be after the end of recovery as determined from its resting rate of movement. If it is nearer the muscle chamber than the calculated position then the R.Q. is less than 1.0 and vice versa (allowance being made for retained CO_2). Obviously the calculation is very much influenced by the accuracy of the base line. On account of the importance of this factor it was customary to observe the muscle for several hours before stimulating and again for several hours afterward. Analysis of the results shows that at least in the majority of cases no possible shift of the base line consistent with the experimental data will suffice to yield a value for the R.Q. equal to 1.0. Some indication of these results can be obtained from figure 4 where the calculated area of each of the rises and falls of the curve above or below the dotted base line has been indicated in $mm.^3$ per gram of muscle. Only in experiments C and F are these two areas so nearly equal that an R.Q. of practically 1.0 is obtained. In 9 other similar experiments tabulated in table 2 the values of $O_2 - CO_2$ are distinctly

greater than the calculated correction so that the R.Q. comes out about 0.92. In 4 other experiments in oxygen the values for the R.Q. were 0.95, 0.69, 0.87 and 0.81 or an average of 0.83 but in this case the correction was larger and the slope of the CO_2 dissociation curve which enters into the calculation is very uncertain.

TABLE 2

The respiratory quotient of the excess metabolism of muscle

Volumes of gas in c. mm. per gm.

1	2	3	4	5	6	7	8	9	10
EXPERIMENT NUMBER	WEIGHT OF MUSCLE	ASSUMED O_2	RISE $O_2 - CO_2$	FALL $O_2 - CO_2$	DIFFERENCE $O_2 - CO_2$	CO_2 OBSERVED	CO_2 RETAINED	CO_2 TOTAL	R.Q.
	mgm.								
A	157	70	16.5	9.1	7.4	62.6	1.8	64.4	0.92
B	100	70	11.0	6.0	5.0	65.0	0.9	65.9	0.94
C	106	30	6.8	6.3	0.5	29.5	0.3	29.8	0.99
D	144	70	22.2	4.4	17.8	52.2	0.8	53.0	0.76
E	144	70	18.0	10.0	8.0	62.0	0.9	62.9	0.90
F	133	20	6.2	5.8	0.4	19.6	0.3	19.9	0.99
G	114	13	5.4	3.0	2.4	10.6	0.2	10.8	0.82
H	134	70	16.5	12.5	4.0	66.0	1.0	67.0	0.96
I	129	70	18.5	11.1	7.4	62.6	1.0	63.6	0.91
J	112	140	45.0	30.0	15.0	125.0	1.4	126.4	0.90
K	107	70	16.0	9.0	7.0	63.0	1.7	64.7	0.92

The sartorius muscle was used in J; otherwise the semitendinosus. The muscle was tetanized for 5 seconds in C and G and for 10 seconds in all other experiments. In J a double period of stimulation was used which accounts for the high values. The low value for oxygen used in G was directly observed by a previous stimulation period over NaOH. Low values for O_2 are used in C and F because the R.Q. so calculated is 0.99. Higher values could not of course make the R.Q. > 1.0. In A, B and K, 0.2 cc. of m/10 HCl was present in the bottom of the respiration chamber. The CO_2 retained by this solution has been added to that retained by the muscle Temperature—14.8°C. In C, F, G and H, 10 per cent CO_2 mixtures in O_2 were used; in other experiments 20 per cent CO_2. The values for the slope of the CO_2 dissociation curve of muscle used in calculating the per cent CO_2 retained were 0.22 and 0.20 cc. per 100 grams muscle per mm. Hg for the 10 per cent and 20 per cent mixtures respectively. Column 6 is the difference between columns 4 and 5. Column 7 is the difference between columns 3 and 6. Column 9 is the sum of columns 7 and 8. Column 9 divided by column 3 gives column 10.

The accuracy of the figures in table 2 for the R.Q. is marred by the fact that except in 1 case a simultaneous observation of the excess oxygen consumed could not be made. This defect was necessitated by the long time necessary for the establishment of perfectly constant diffusion gradients and hence of a constant base line. Except in particular cases, as described in the legend for table 2, a value for the excess oxygen consumption of 70 ×

10^{-5} cc. per gram was assumed for the purpose of calculation. Comparison of this value with the curves in figure 3 shows that for 10 seconds' stimulation this represents about the upper limit (except for the data of curve *1* which, however, is altogether exceptional). Of the figures for excess oxygen per 10 seconds of stimulation from 24 stimulation periods, other than those plotted in figure 3, only 3 give values of 0.07 cc. per gram or over; 3 others lie between 0.05 and 0.07, and 17 are less than 0.04 cc. per gram. The figures for the R.Q. in table 2 are, therefore, too high on this account rather than too low.

All the determinations of the R.Q. of the excess metabolism have been made upon muscles from frogs kept at a temperature just above freezing for a period of at least several days. In spite of the well recognized imperfections in the method the conclusion seems justified that in muscles such as these it is very probable that something other than carbohydrates is being burned in small amounts. There are, however, many theoretical difficulties in drawing any certain conclusions from R.Q.'s even in the intact organism and in the isolated muscles these difficulties are in some respects greater. Possibilities are always present of accumulation of acid or of alkali, incomplete oxidation, etc. The method used by Meyerhof involving direct determination of preformed carbonates is not open to this objection.

5. *The time course of the gas exchange.* One of the striking characteristics of these experiments in high CO_2 mixtures is the initial rise indicating that the oxygen goes in before the CO_2 goes out. There can be little doubt from the following considerations that this is due to diffusion factors. In studying the absorbtion of CO_2 by muscles at different tensions arrangements have been made to change rapidly the tension of CO_2 in the respiration chamber and to observe the rate of diffusion of CO_2 by the movements of the index drop. Such diffusion curves are approximately exponential, the CO_2 accumulating in the muscle according to the equation $y = y_o e^{-\frac{t}{16}}$. This signifies that, on the average, $1 - \frac{1}{e}$ or 63 per cent of the diffusion is complete in 16 minutes. Consider now a piece of tissue in which at time 0 a given amount of oxygen is used up and an equal amount of CO_2 is produced. CO_2 will begin to diffuse out according to the equation given above. Oxygen will begin to diffuse in according to a similar exponential equation but with a different time constant. The rate of diffusion of oxygen should be proportional to the diffusion coefficient for that gas and inversely proportional to the solubility of the gas in the tissue. The inverse of the solubility is the change in tension which will be produced by a given change in content, i.e., the diffusion gradient. Krogh (1919) has shown that the diffusion constant of CO_2 is 30 times that of O_2 at 20°C.[2] How-

[2] On rereading Krogh's (1919) paper I find that he states that the diffusion constant of CO_2 is 35.7 (not 30) times that of O_2. This was based, however, on a single determination which the author thought might be too high.

ever, the solubility of oxygen in water at 15°C. is 0.034 cc. per gram per atmosphere pressure while the solubility of CO_2 in muscle in 10 per cent CO_2 mixtures at 15° is (slope of the dissociation curve) 0.22 cc. per 100 grams per mm. Hg (as shown above) or 1.67 cc. per gram per atmosphere. The diffusion gradient for the oxygen will, therefore, be 1.67/ 0.034 or 49 times as great as for CO_2 for the same change in content.[3] Oxygen may, therefore, be assumed to diffuse into the muscle according to the equation

$$y = y_o e^{-\frac{t}{16 \times \frac{30}{49}}} = y_o e^{-\frac{t}{9\frac{7}{7}}}.$$

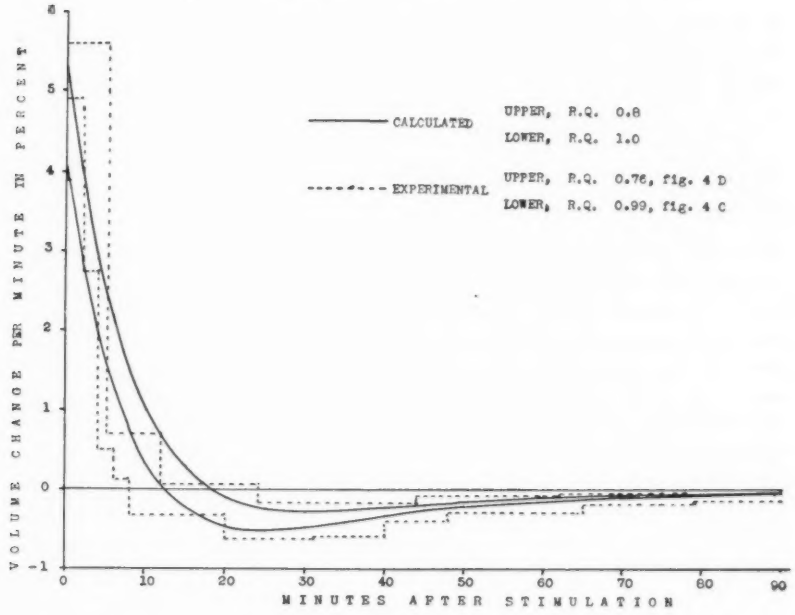

Fig. 5. Calculated and experimental values of $O_2 - CO_2$ per minute or $\frac{d}{dt}$ plotted against time. Ordinates represent rates in per cent of the total excess oxygen per gram per minute.

[3] According to Graham the diffusion rates of gases should be inversely proportional to the square roots of their densities. On this basis O_2 should diffuse $\sqrt{\frac{44}{32}}$ or 1.173 times as fast as CO_2 instead of $\frac{16}{9.7}$ or 1.63 times (cf. Taylor, Physical Chemistry, p. 934).

Taking V_{O_2} and V_{CO_2} as the total volume change of O_2 and CO_2 respectively, when diffusion is complete (y_o) then the volume, V, of $O_2 - CO_2$ outside the muscle at time t will be given by

$$V = V_{O_2} e^{-\frac{t}{9.7}} + V_{CO_2} - V_{CO_2} e^{-\frac{t}{16}}$$

and the rate of change of volume will be

$$\frac{dV}{dt} = \frac{V_{CO_2}}{16} e^{-\frac{t}{16}} - \frac{V_{O_2}}{9.7} e^{-\frac{t}{9.7}}$$

If V_{O_2} is taken as 100 then V_{CO_2} will be 100 or 90, etc., according to the R.Q. *The oxygen will, therefore, diffuse in more rapidly than CO_2 will diffuse out because its solubility is so low that its diffusion gradient is great enough to more than compensate for the greater diffusion coefficient of CO_2.*

The graphs of figure 5 will show how well the experimental curves can be explained on this basis. The solid lines are drawn according to the equation for $\frac{dV}{dt}$ given above; the upper one represents the rate of volume change when the R.Q. is 0.8 and the lower one when the R.Q. is 1.0. In the latter case the area above the base line is exactly equal to the area below the base line. The dotted graphs are taken from experiments C and D in figure 4 and are plotted as percentages of the total excess oxygen values which were given in the calculation of these experiments in table 2. The calculated curves are plotted in the same units if V_{O_2} is taken as 100. The upper experimental graph calculates out to an R.Q. of 0.76 and should, therefore, approximate to the upper calculated graph. The lower experimental graph calculates out to an R.Q. of 1.0 and more nearly resembles the lower calculated curve. Too much stress should not be laid upon the exact coincidence of the calculated and the experimental curves, partly because the experimental curves are plotted as per cent of an assumed excess oxygen value, and partly because the time constant for the exponential equation must vary from one muscle to another. The actual values of this constant in 8 experiments as found experimentally were 26, 14, 7, 8, 17, 10, 29, 21, the fresh weight of the muscles varying from 76 to 130 mgm. Moreover no allowance has been made, in the comparison, for the small amount of CO_2 retained in the muscle. Nevertheless the graphs of figure 5 may be taken as convincing evidence that the relative rates of diffusion of O_2 and CO_2 explain an important part of the experimental results.[4]

The question may be raised whether these same relative rates of diffusion of oxygen and CO_2 have any physiological significance in problems con-

[4] A similar analysis has been made of the time course of the gas exchange in nerve. (Fenn. 1927. Journ. Gen. Physiol., xi, no. 2.)

cerning the gas exchange in the capillaries during muscular exercise. Theoretically the blood which leaves a given capillary immediately after the muscle fibres along its course have contracted should contain a deficiency of oxygen and should be abnormally alkaline (assuming no equally prompt change in blood flow). Blood leaving the same capillary shortly afterwards will contain excess carbon dioxide and will be abnormally acid. However, the diffusion is so rapid across the short distances involved in a single capillary field, that the duration of the initial alkalinity would doubtless be measured in seconds and would be too brief to demonstrate experimentally. It is not surprising, therefore, that by virtue of the great efficiency of the respiratory center the first effect of muscular exercise is an increase in the R.Q., rather than a decrease as in isolated muscle. In addition, it should be mentioned that in mammalian muscles the relative rates of diffusion described in this paper would not apply quantitatively. In the first place the CO_2 tension in the muscles is less than 10 per cent which would increase the solubility of CO_2. The more the CO_2 accumulates in the tissues the less will be its solubility and so the greater the diffusion gradient for a given CO_2 production. Moreover the presence of muscle hemoglobin will greatly increase the solubility of oxygen. Possibly some of the significance of muscle hemoglobin lies in its ability to equalize the solubilities of O_2 and CO_2 thus avoiding an initial though brief blood alkalinity after contraction.

SUMMARY

1. With a sufficiently delicate respirometer it is possible to measure the extra oxygen consumed by a small frog muscle in recovering from a 5 to 20 seconds' tetanus, recovery being complete in 30 to 40 minutes.

2. The excess oxygen consumption is nearly, but not quite, doubled when the duration of the tetanus is doubled.

3. The lactic acid formation for 10 seconds' stimulation, as measured by Embden, would require about 5.7 times as much oxygen for its combustion as the muscles were observed to consume for a similar period of stimulation.

4. From known rates of oxygen consumption of muscles and values of $O_2 - CO_2$ per minute in atmospheres of 10 per cent CO_2 a value of 0.92 for the R.Q of resting muscles is arrived at, after making a small correction for the small amount of CO_2 retained at this tension.

5. By similar methods the R.Q. of the excess metabolism is found to be about 0.93 on the average.

6. After stimulation the excess oxygen is observed to diffuse into the muscle considerably before the CO_2 begins to diffuse out.

7. This peculiar time course of the gas exchange is quantitatively explained by the high solubility of CO_2 in the muscle which is more than enough to compensate for its greater diffusion constant.

I am indebted to Mr. W. B. Latchford for invaluable assistance during this investigation and to Mr. F. Metildi, medical student, for a number of preliminary experiments.

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THE REGULATION OF RESPIRATION

XIII. Effects of Changes in Oxygen Content of Artificially Administered Gaseous Mixtures on Expired Carbon Dioxide and Oxygen as Studied with Continuous Electrometric Methods[1]

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It is a fair assumption that variations in pulmonary ventilation are a result of changes in equilibrium in the controlling neuro-mechanism and its immediate pericellular environment. That being true, synchronous records of the behavior of this mechanism as indicated by respiratory movements and of the chemical changes within it and its environment should constitute a profitable method of study. In such a study we believe that acute and reversible disturbances of relatively short duration are particularly worthy of analysis. Herein lie the advantages of continuous methods of recording such pertinent changes as acidity of the blood and body fluids, (Gesell and Hertzman, 1926), volume-flow of blood (Gesell and Bronk, 1926) and expired carbon dioxide and oxygen (Gesell and McGinty, 1926).

This paper is the first of a series of papers in which we use our continuous electrometric methods of recording expired oxygen and carbon dioxide. The method is essentially the same as previously described. Changes in oxygen content of the expired air are followed by means of a specially devised thermopile vessel and potentiometer which register variations in temperature of a bunsen flame supplied under constant conditions with city gas and expired air. An increase in oxygen content of the expired air leads to a better combustion of the gas and a greater liberation of heat to the stream of water flowing through the flame and thermopile vessel. There is a resulting increase in E.M.F. delivered by the thermopile vessel which is registered mechanically as an upstroke on the smoked records. To avoid irregularities in E.M.F., which occasionally developed as a result of bubble formation and accumulation in the thermopile vessel, the distilled water flowing through the vessel is regularly cooled to approxi-

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mately 12°C. and exhausted in the Mariotte bottle with a water suction pump for approximately twenty minutes just prior to the experiment. To avoid shifts in the absolute E.M.F. values resulting from variations in the composition of city gas throughout the day, a supply of gas is stored in an 80-gallon boiler tank under pressure with a gas pump sufficient to last for three or more experiments. The gas is relayed through an automatically filling spirometer which delivers the gas at approximately constant pressure to the second constant pressure spirometer.

The method for recording changes in expired carbon dioxide has also been improved by adding stock phthalate buffer solution (Clark and Lubs) of pH 5.0 to the saturated KCl to a concentration of 0.0005M. This has the advantage of reducing the sensitivity of the method, permitting a more convenient registration of changes in carbon dioxide tension and of establishing a more linear relation between carbon dioxide tension and E.M.F. developed. As a result the records are more easily interpreted.

In this and the following paper the oxygen and carbon dioxide methods are calibrated separately by delivering various oxygen and carbon dioxide mixtures of known composition to the bunsen burner and electrode respectively. This method has been decidedly improved in the third and following papers. The results of the first two papers are, therefore, not as quantitative as those following. Nevertheless, they bring out certain facts that seem worthy of record.

Results. Some twenty experiments were performed. The discussion of results is limited to the published figures obtained from six of these experiments. The data were obtained on dogs during artificial ventilation. The ventilation is positive and constant. Changes in expired oxygen and carbon dioxide, therefore, give direct information on changes in the total amount of oxygen absorbed and carbon dioxide eliminated. A characteristic example of the behavior of the dog to the administration of a gaseous mixture low in oxygen (4 per cent) is seen in figure 1A. The records from above down are timed in second and five-second intervals, signal, expired carbon dioxide, mean blood pressure, expired oxygen, artificial ventilation, and abdominal movements. For convenience a horizontal bar corresponding to a five-minute period divided into one minute intervals is added to each figure. Downstroke indicates decreasing carbon dioxide and oxygen content of the expired air (total mixed sample of alveolar air and dead space air). Upstroke indicates increasing carbon dioxide content and oxygen. The vertical brackets marked CO_2 and O_2 correspond to a change of 1 per cent in expired carbon dioxide and oxygen respectively. The height of the CO_2 bracket varies with the diameter of the spindle on the potentiometer employed for recording and the sensitivity of the electrode. The corresponding points with respect to time are marked on each record and the procedures signalled and labeled.

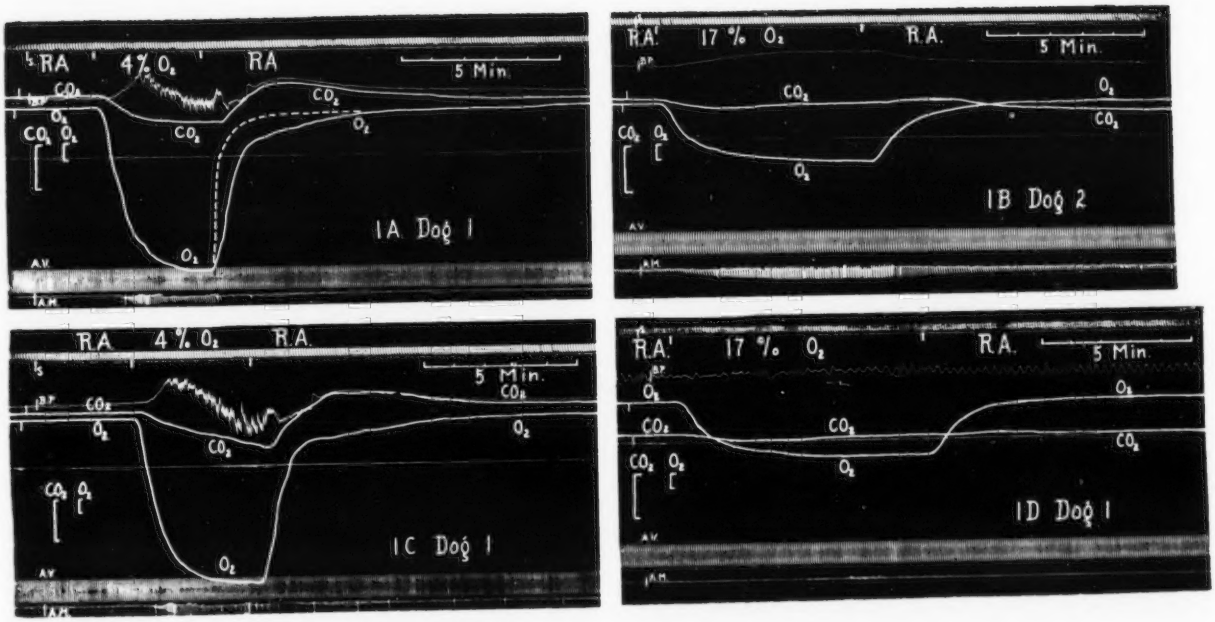
RA 4% O2 RA
5 Min.
1A Dog 1
R.A. 17 % O2 R.A.
5 Min.
1B Dog 2
R.A. 4 % O2 R.A.
5 Min.
1C Dog 1
R.A. 17 % O2 R.A.
5 Min.
1D Dog 1

Fig. 1

At the beginning of record 1A the dog was receiving room air and during this period expired carbon dioxide and oxygen remain constant. On the administration of a 4 per cent oxygen mixture the expired oxygen suddenly falls. At the same time expired carbon dioxide drops, rapidly at first and then more slowly up to the readministration of room air. Now the expired oxygen suddenly increases and also the expired carbon dioxide. The oxygen curve slowly approaches the initial level obtaining during the administration of room air at the beginning of the record. In the mean time the expired carbon dioxide overshoots its normal initial value and then slowly returns to that level.

The interpretation of the carbon dioxide record is simple as it gives a direct picture of the amount of carbon dioxide expired. The picture is slightly improved, however, by making mental correction for the lag of the carbon dioxide electrode. Since equilibrium is not immediately established the curve falls behind the actual occurrences. The correction for this becomes significant only in the more rapid and in the shorter changes in carbon dioxide tension. The initial drop in carbon dioxide on the administration of low oxygen is, therefore, slightly steeper than the curve indicates. In the oxygen record there is an inverse relation between the amount of oxygen expired during the administration of any gaseous mixture and the amount retained. A drop in the oxygen record, for example during a prolonged administration of room air in figure 3B, means an increased absorption of oxygen. Where rapid changes in expired oxygen occur the lag in the oxygen method is also a significant factor. The dotted curve in figure 1A is the shape of an in-vitro calibration curve showing the lag of the method in changing from a gas of low oxygen content to another of higher oxygen content, corresponding to the changes in the in-vivo observation. In comparing the continuous in-vivo record with the in-vitro calibration curve it will be noted that the in-vivo record rises more slowly at first and then more rapidly than the calibration curve and finally runs in a horizontal direction. The horizontal level indicates the amount of oxygen retained during the administration of room air. The lower position of the in-vivo curve indicates that the animal is retaining an increased amount of oxygen. Barring the diluting effects of the lung volume on the shape of the in-vivo curve on readministration of room air the area of the crescent confined by the two curves indicates the amount of excess retention of oxygen. Corresponding with this period of oxygen over-retention there is a period of carbon dioxide overshooting, in which elimination is considerably above normal. As oxygen retention approaches normal once more carbon dioxide expired also returns to normal. The complete return of expired gases reflects the reversibility of the respiratory and circulatory processes and the relatively slight degree of tissue injury in these particular instances. Though the experiments were not planned

to show directly the impairment of oxidations during the period of low oxygen administration, the behavior of the animal during readministration of room air indicates that anaerobic metabolism had been definitely increased. It is a fair assumption that the temporary excess gaseous exchange is compensatory for the preceding low exchange.

Figure 1C is another observation on the same animal later in the course of the experiment. The effects are more severe, as suggested by the changes in mean blood pressure. The decrease in carbon dioxide elimination is greater and more progressive and the excess oxygen retention and carbon dioxide overshooting during recovery are greater than in figure 1A. The greater depression of circulation is probably a contributing factor to the greater depression of gaseous exchange. The circulatory factor must be considered in the interpretation of gaseous exchange, as it is usually present to some degree.

The fundamental changes in expired gases, illustrated most strikingly in figure 1A and C, will be seen to appear in all of the records where recovery from administration of low oxygen was good. See figures 1A, B, C and D, 2A, B, C, D and E and 4D and E. To be sure variations in the extent of the changes occur from animal to animal. In analyzing the records an alkaline basal drift of the carbon dioxide electrode will be evident in a few of the records. If the oxygen record remains horizontal over a long period and the carbon dioxide elimination apparently diminishes, the electrode is drifting. Where overshooting is small it may not be obvious unless correction is made for this drift.

Glancing through the figures it is noted that in general the larger the changes in oxygen administered the greater the reaction. Observations such as 1A and C, therefore, bring out the salient points more clearly. It is nevertheless interesting to study the response of the various mechanisms associated with changes in expired gases by a graded administration of oxygen in the same animal. In this way, too, we arrive at suggestions on the sensitivity of the chemical mechanisms involved. We find that one animal may show a relatively large reduction of carbon dioxide elimination for a given reduction of oxygen administered, as compared to another animal (see e.g., figs. 2D and E, in which a 9 per cent mixture of oxygen was administered). On the other hand, when the oxygen administered is progressively changed in any single animal the carbon dioxide eliminated shows progressive changes (see figs. 2A and B, which illustrate effects of 4 per cent, 13 per cent and 17 per cent oxygen mixtures on carbon dioxide elimination in one animal; figs. 2C and D, which show effects of 17 per cent, 13 per cent and 9 per cent oxygen mixtures in another animal, and 1D, 4D, 4E and 1A, which show effects of 17 per cent, 13 per cent, 9 per cent and 4 per cent oxygen mixtures in still another animal). It is evident in these records that changing from room air to a 17 per cent oxygen mixture

and back again to room air produces characteristic changes in the expiratory gases. In figures 3A and D the oxygen percentage is reduced following room air only to 19 per cent but still with positive effects. Finally, in figures 3C and E a still smaller change in oxygen administration was tried on another animal—from room air to a 20 per cent oxygen mixture. The results in figure 3C appear irregular but they are explained by the preceding figure, 3B, taken from the same animal. This record shows periodic changes in expired carbon dioxide and oxygen presumably related

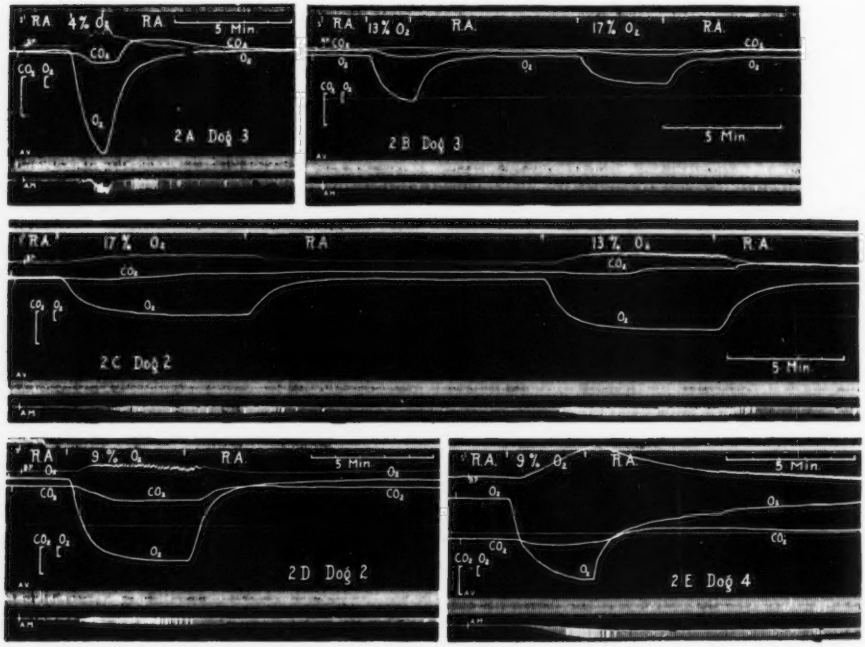

Fig. 2

to periodic disturbances in circulation or oxidations. By following the waves through, a high oxygen retention is found to correspond to a high carbon dioxide elimination and the reverse. This periodicity present before the administration of 20 per cent oxygen undoubtedly continued through the administration and modified the results. The findings on the effects of the higher oxygen mixtures agree with what is known, through the work of Schneider, Truesdell and Clark, (1924) and others, on the sensitiveness of the response of man to minute reductions in barometric pressure.

Comparing the effects of short and long administrations of low oxygen an initial reduction in carbon dioxide elimination and a final carbon dioxide overshooting on readministration of room air will be found common to all. But in the more prolonged administrations the progressive decrease in carbon dioxide elimination gives way to a very slowly increasing carbon dioxide elimination, merging into the carbon dioxide overshooting on readministration of room air. This is seen in figures 1B and D, 2B, 2C and 2E, and 3A. One of the first explanations which occurred to us was the accu-

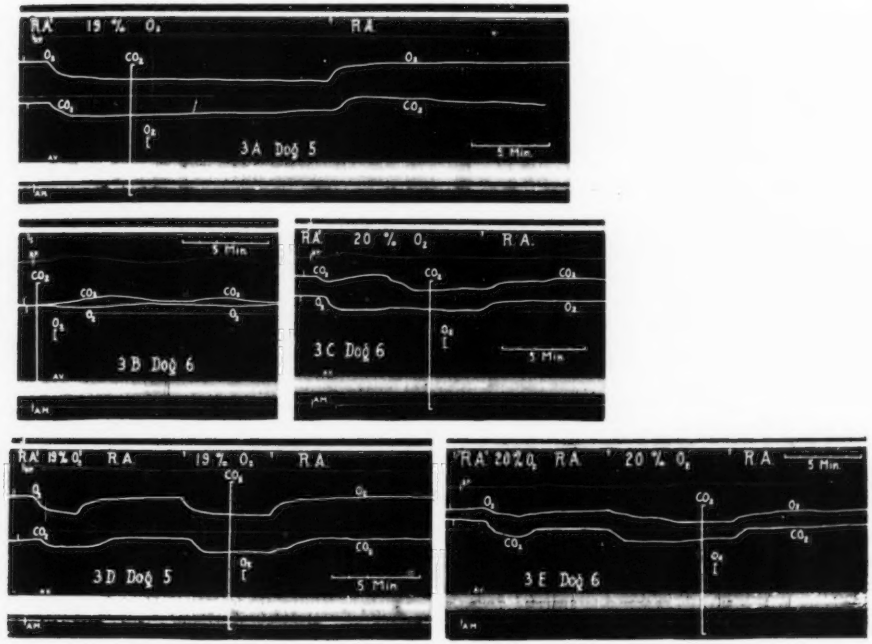

Fig. 3

mulated acid effect of augmented activity of the respiratory muscles during the lowered oxygen administration. Conceivably, this should at least be a contributory factor, yet, as will be seen in figure 1D, in which the dog remained continuously apneic (no abdominal movements), it may not be the only factor for carbon dioxide elimination increased during the period of lowered oxygen administration. We are inclined to turn to the coordination of the dual function of hemoglobin as another factor. A lowered oxidation of the hemoglobin in the lungs should lead to lowered

carbon dioxide tension, due to increased free base of the blood, and, therefore, a retention of carbon dioxide by the blood. But the blood continuing to flow through the tissues takes up more and more carbon dioxide and fixed acids, and the carbon dioxide tension again increases and with that an increase in carbon dioxide elimination.

Obviously a detailed explanation of the changes in expired gases requires a quantitative study of several factors. Reasons are sufficient for believing that volume-flow of blood, variations in the coördination of the dual function of hemoglobin, variations in muscular activity, variations in oxidations, and aerobic and anaerobic acid metabolism are all important. Yet it is not a simple problem to evaluate the significance of each. If the volume-flow of blood is impaired by severe reduction in alveolar oxygen, the oxidations in the tissues are not only impaired by the lowered oxidation of the hemoglobin but by the reduced flow as well. The hampered flow of blood reduces the transport of acid from the tissues and finally a reduced flow of blood through the lungs reduces the elimination of carbon dioxide there. This is indicated in figure 4C, where fatal circulatory failure occurred after administration of a 4 per cent oxygen mixture. In an effort to revive the animal, 120 cc. of glucose solution were injected intravenously with a momentary improvement of the circulation. There was a marked increase in carbon dixoide elimination. For further information on the relation of blood flow to expired gases see the following paper on hemorrhage and reinjection.

That the changes in expired gases are related to the oxidative processes in the tissues is only reasonable to expect, and we have pointed out in the description of figure 1A and 1C that the carbon dioxide overshooting on readministration is partly a function of oxidative recovery, in these instances of excess oxidations. In contrast we may note the behavior of a deteriorated animal towards the close of an experiment (see fig. 4A). Oxygen consumption during the administration of room air is poor and the mean blood pressure is low. The reaction to the administration of 13 per cent oxygen is quite different from that in vigorous animals. The blood pressure falls and pulmonary ventilation diminishes. On readministration of room air pulmonary ventilation increases, mean blood pressure continues to fall, though not as rapidly. The usual hyperoxygen consumption is missing; as a matter of fact, it fails to return to the level of that of the preceding room air administration. Corresponding to the impaired oxidations is the absence of carbon dioxide overshooting. A second administration of 13 per cent oxygen produces a further impairment of oxidation and a further reduction in carbon dioxide elimination during the subsequent administration of room air. At this time the animal is very sensitive to reduction in oxygen administration, and in the next observation (9 per cent O_2) the mean blood pressure and carbon dioxide

elimination drop sharply. On readministration of room air the oxygen curve rises abruptly to a high level and remains thus for several minutes, indicating very low oxygen retention. Carbon dioxide elimination does not increase for several minutes until the oxygen curve breaks. That the increased carbon dioxide elimination is a result of a sudden improvement of the oxidative function in the tissues is very probable, yet the record does not constitute a clean cut demonstration. Most probably the moment that oxidations improved in the heart, circulation improved as well. This point is made simply to emphasize the difficulty of evaluating the significance of the various factors involved in gaseous exchange. The same results are brought out more sharply in figure 4B, in which a 4 per cent oxygen mixture is administered to an animal in a somewhat better condition than the animal of figure 4A. There is a big fall in blood pressure and carbon dioxide elimination. On readministration of room air the oxygen curve rises sharply, indicating low oxygen consumption. Carbon dioxide elimination remains unimproved for several minutes. Suddenly oxidation, circulation and carbon dioxide elimination improve. In the following record, 4C, oxidations fail to recover on readministration of room air.

Reviewing the records with respect to the activity of the respiratory muscles, as indicated by the records of abdominal movements (the writing point was connected by vertical suspension to the point of greatest motion on the abdomen), the general effect of low oxygen administration will be found to be excitatory and subsequent administration of room air the reverse. In most instances slight respiratory movements were augumented or apnea was interrupted by low oxygen. In some instances apnea was not interrupted presumably due either to more excessive ventilation or lesser stimulation. An exception to excitation is found in figure 4A where the animal was in very bad condition towards the close of the experiment. The respiratory movements, therefore, on the whole show a rough inverse relation to alveolar oxygen pressure and the rate of oxidations. But exception to this inverse relation to rate of oxidation even when the animal was in good condition is noted in figures 1A and C in which abdominal movements temporarily increase on readministration of room air. Most probably oxidations are increasing and decreasing as respiratory movements respectively increase and decrease. This same parallel relation comes out more clearly following reinjection in hemorrhage experiments and will be discussed in greater detail there.

Looking at the problem from the acid point of view, the records indicate a direct relation between respiratory movements and acid accumulation in the body. This hinges on the assumption that with the diminution in oxygen supply the aerobic acid metabolism gives way to an anaerobic acid metabolism. The records show that low oxygen administration

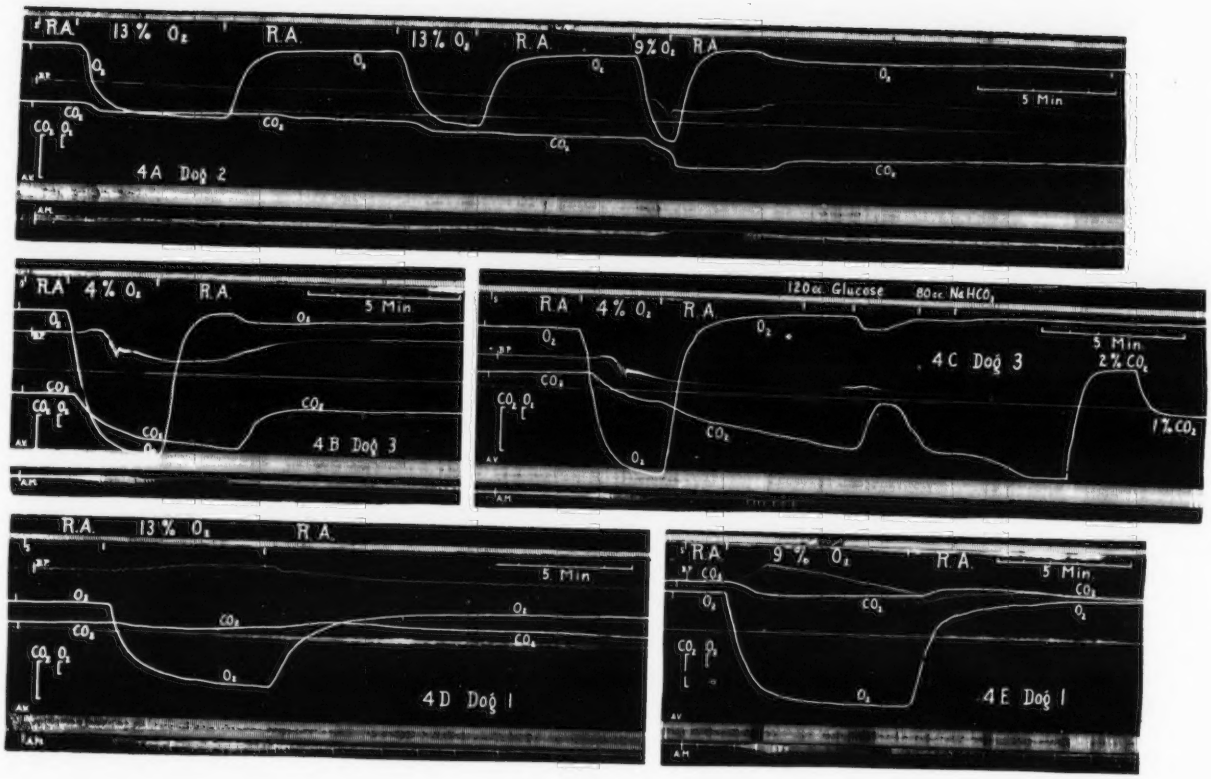
R.A. 13% O2 R.A. 13% O2 R.A. 9% O2 R.A.
5 Min
4A Dog 2
R.A. 4% O2 R.A.
5 Min
4B Dog 3
120cc. Glucose 80cc. NaHCO3
R.A. 4% O2 R.A.
5 Min.
2% CO2
1% CO2
4C Dog 3
R.A. 13% O2 R.A.
5 Min
4D Dog 1
R.A. 9% O2 R.A.
5 Min
4E Dog 1

Fig. 4

leads to lowered acid elimination, and granting that a broken coördination of the dual function of hemoglobin hampers the transport of acid from the tissues some of this accumulated acid remains in the tissues as well as in the blood. On readministration of room air oxidations are improved, lactic acid disappears, the normal coördination of the dual function of hemoglobin returns, acid is liberated in super abundance through the lungs and the tissues presumably turn more alkaline. The respiratory behavior with changes in oxygen administration fits in with the acid mechanism of respiratory control. This has been further supported by the experiments on the effect of changes in oxygen administration by artificial ventilation on the acidity of the blood (Gesell and Hertzman, 1927). Reducing the oxygen content of the gaseous mixture administered below that of room air increases the alkalinity of the blood. Subsequent readministration of room air is accompanied by a temporary increase in acidity considerably above the normal level for room air. The lowered acid output during the administration of low oxygen then corresponds with increased alkalinity of the blood. The carbon dioxide overshooting corresponds with the acid overshooting in the pH experiments. These findings are in agreement with an earlier statement by one of us, Gesell (1925) in relation to the control of ventilation by variations in oxygen pressure—that other things remaining constant, the more acid the blood the better the transport and elimination of acid, and the more alkaline the tissues.

SUMMARY

The effects of changes in oxygen pressure of gaseous mixtures on expired carbon dioxide and expired oxygen were studied with continuous electrometric methods.

The gaseous mixtures were administered in constant volume by artificial ventilation during pneumo-thorax. The changes in expired gases were recorded directly on smoked paper along with mean blood pressure and respiratory movements.

A temporary reduction of the oxygen content of the gaseous mixture below that of room air reduced the carbon dioxide content of the expired air. Readministration of room air temporarily increased the carbon dioxide elimination above that of the preceding room air administration. This carbon dioxide overshooting was found to correspond with a synchronous excess oxygen retention.

The greater the reduction of oxygen content of the administered air the greater was the reduction of carbon dioxide eliminated and the greater were the carbon dioxide overshooting and excess oxygen retention on readministration of room air.

Reduction of the oxygen content from that of room air to a 20 per cent oxygen mixture elicited a small but typical effect in gaseous exchange.

With prolonged administration of low oxygen the decreasing carbon dioxide elimination gave way to slowly increasing elimination, which merged with the acid overshooting on readministration of room air.

It was suggested that the decreased carbon dioxide elimination during the administration of low oxygen is a result of impaired oxidations and a broken coördination of the dual function of hemoglobin, which hampers the transport of acid from the tissues to the lungs and its elimination in the lungs.

The carbon dioxide overshooting and excess oxygen retention on readministration of room air were explained by compensatory oxidations and improvement of the coördinated function of hemoglobin in the transport and elimination of carbon dioxide.

The carbon dioxide overshooting of the expired gas experiments corresponds with the acid overshooting of the blood on the readministration of room air in the blood acidity experiments.

The results appear in agreement with an acid mechanism of respiratory control.

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THE REGULATION OF RESPIRATION

XIV. The Effects of Hemorrhage and Reinjection on Expired Carbon Dioxide and Expired Oxygen as Studied with Continuous Electrometric Methods[1]

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The factors influencing the composition of expired air are rather complex and numerous. Of these the composition of the gaseous mixture administered, rate and nature of the metabolism in the tissues, transport of the gases between the lungs and tissues and volume of gas administered all exert their various effects. In our effort to evaluate the significance of the factors involved we are beset with the difficulty of introducing only one variable at a time. In the intact animal the composition of the administered gas alone is under accurate control. By establishing pneumothorax and administering artificial ventilation the volume of gas is likewise controlled and the problem is enormously simplified. For the present, however, this appears to be the limit of accurate control of any single factor.

In the preceding paper (p. 323) we have attempted a preliminary analysis of the effects of composition of the administered gases. By varying the oxygen content of the gaseous mixture we hoped to vary the degree of oxidation of hemoglobin in the lungs and thereby show the effects of a broken coördination of the dual function of hemoglobin in the pulmonary circulation by following synchronous changes in carbon dioxide elimination.

It was found that reduced oxygen administration promptly reduced the elimination of carbon dioxide at the lungs, thus illustrating in the in-vivo experiment the significance of oxidation of hemoglobin in the expulsion of carbon dioxide. But a poorly oxidized blood arriving at the tissues provides less oxygen to the tissues and leads to a second disturbance in the coördination of the dual function of hemoglobin by a failure to provide the necessary base for the transport of acid from the tissues to the lungs. The effects of hampered transport of acid to the lungs are, therefore, added to the hampered elimination at the lungs. Though the results of such experiments are largely interpretable in terms of coördination of the dual function of hemoglobin, it must be remembered that the degree of

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impaired coördination of function is directly dependent on the degree of impaired oxidations in the tissues. The complications resulting from the interdependence of the various processes are hard to circumvent. The function of hemoglobin is dependent on the behavior of the tissues, and the behavior of the tissues is dependent on the function of hemoglobin. It is, therefore, reasonable to believe that a broken coördination of the function of hemoglobin, regardless of how it is brought about, affects the metabolism of the tissues.

In the present experiments we have studied the effects of volume-flow of blood on the composition of the expired air. Here again it must be apparent that the transport of gases as affected by the changes in volume-flow will influence oxidations in the tissues and these changes will in turn determine the transport of gases from the tissues to the lungs.

The methods used are similar to those described in the preceding paper. Room air is continuously administered at a constant volume. Changes in volume-flow are elicited by hemorrhage from the femoral artery and reinjection into the femoral vein. Sufficient heparin is added to each sample of blood to avoid clotting before reinjection. In some instances isotonic saline solution was injected as a substitute for blood.

Results. Ten experiments were performed. Observations from seven of these are described and illustrated in three accompanying figures. The same general description of figures given in the preceding paper holds. The amount of hemorrhage in cubic centimeters and per cent of body weight and duration of hemorrhage and injection, and the weight of the dog are indicated on each record.

Figure 1B shows typical changes in the expired gases. The dog was bled 270 cc. (2.25 per cent body weight) within a period of three minutes. After the initial fall, mean blood pressure was constantly maintained during the period of depleted blood volume. Expired oxygen (decreased oxygen retention) was increased and carbon dioxide elimination promptly decreased. In about two minutes a constant expiratory exchange was established. Subsequent injection of the drawn blood plus 25 cc. of saline solution elicited increased oxygen retention and increased carbon dioxide elimination. The oxygen curve is seen to fall considerably below the initial level during the period of normal blood volume and to gradually rise to the normal level at the very end of the record. Corresponding to these changes in the oxygen record the carbon dioxide curve shows an initial overshooting with a gradual return to normal. Actually the record falls below the normal level but this is accounted for by the alkaline drift of the manganese dioxide electrode evident at the beginning of the record where the condition of the animal was presumably constant. The expiratory gas records in short show considerable resemblance to those elicited in the preceding experiments by the administration of air poor in oxygen.

The expiratory exchange during depleted blood volume indicates hampered transport of gases and impaired oxidations. The expiratory exchange on reinjection supports these indications. The increased carbon dioxide elimination and oxygen retention presumably are a compensatory function of the oxygen deficiency and acid accumulation of the preceding period of lowered volume-flow. The results, as in the preceding paper, are in-

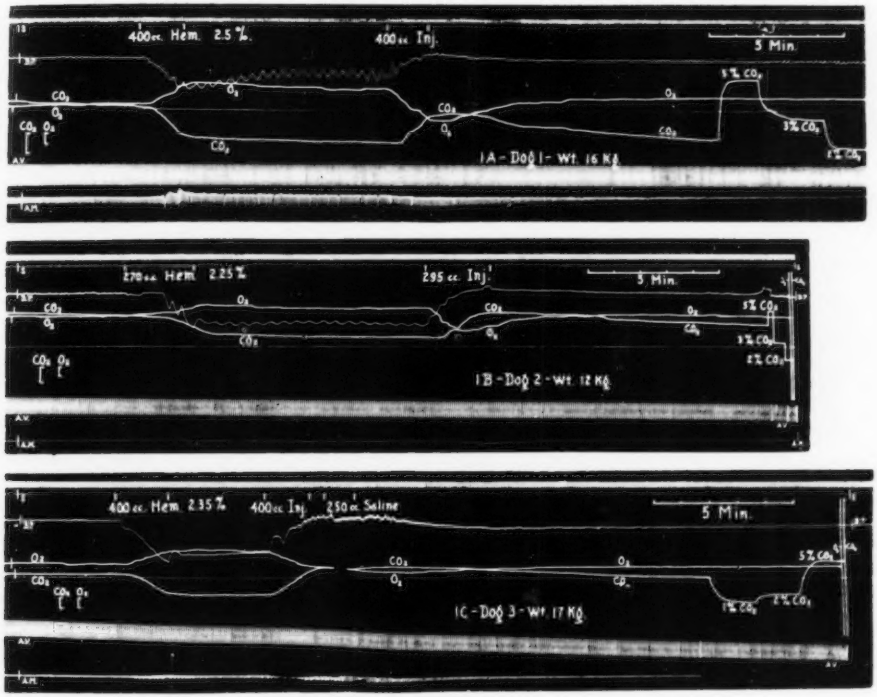

Fig. 1

terpretable in terms of associated changes in gaseous transport and gaseous metabolism.

The findings in figure 1A present slight variations. Though the hemorrhage is somewhat greater (2.5 per cent of the body weight) the effect on mean blood pressure is smaller; nevertheless the effects on expiratory exchange are more pronounced. Of interest is the gradual increase in mean blood pressure during depleted blood volume accompanied by increased oxygen retention. Carbon dioxide elimination shows a corresponding increase which is apparent if the basal alkaline drift of the electrode during

the period of normal blood volume is extended. Reinjection brings on the usual hyperoxygen retention and carbon dioxide elimination, but the oxygen curve shows a rapid recovery rising above the normal initial values —indicating that oxygen consumption was more or less permanently hampered by the period of depleted blood volume. Carbon dioxide elimination is similarly affected. In figure 2A the hemorrhage is still greater (3.6 per cent of the body weight). The effects on expiratory exchange are likewise great.

In figure 1C hemorrhage elicited the usual results. On reinjection of the blood plus 250 cc. saline solution the oxygen consumption is permanently increased above the pre-hemorrhage value. A similar effect of increasing the blood volume with saline solution is seen in the early part of record 3B just before the 450 cc. hemorrhage. Expired oxygen and carbon dioxide show corresponding changes. Hemorrhage impaired the expiratory exchange. Note how closely the carbon dioxide record follows the mean blood pressure tracing. The blood is replaced by 450 cc. of saline solution which improves the gaseous exchange. Increasing the blood volume still further by reinjection of the drawn blood slightly increases oxygen consumption and carbon dioxide elimination, though the effect is transitory. The same momentary effects of hydremic plethora appear on the second injection following hemorrhage in figure 3C.

In figure 3A typical but relatively small effects appear though the hemorrhage is the largest of the series (5.79 per cent body weight). (The gradient of the artificial ventilation is of no significance. It is simply a record of a leak in the dead end of the pump.) Reinjection plus additional saline to a total of 930 cc. fails to bring the expiratory exchange back to normal. A semi-permanent impairment of the circulatory system or oxidations would account for such results and illustrate again the interdependence of circulation and of tissue metabolism in establishing gaseous exchange.

Though the experiments were not designed to bring out the sensitivity of the respiratory mechanism to changes in blood volume, but rather to establish other principles, it is not amiss to review the results in that connection. It will have been noted that the hemorrhages on the whole have been large—varying from 2.25 per cent to 5.79 per cent of the body weight. With hemorrhages of such magnitude great changes in the expired gases are to be expected. Following the hemorrhages through from experiment to experiment a rough relation between extent of hemorrhage and expiratory gases seems to hold. Figure 3A, however, stands out as an exception. The animal in this experiment for some reason or other was relatively insensitive to blood volume changes. The variation in susceptibility to hemorrhage in different animals is well known and has been previously pointed out on other occasions (Gesell, Blair and Trotter, 1922; Gesell and Moyle, 1922a, Gesell, Capp and Foote, 1922; Gesell, Foote and Capp, 1923).

The relation of the extent of hemorrhage to expiratory exchange is more easily demonstrated by graded hemorrhage on the same animal. Two such experiments on two different dogs are shown in figures 2B and C. In figures 2B there are four 100 cc. hemorrhages, each amounting to 0.74 per cent of the body weight. It will be noted that the effects of each hemorrhage become progressively greater. In figure 2C the animal is even more sensitive to hemorrhage. There are decided changes in the expired gases following hemorrhages each equal to 0.50 per cent of the body weight.

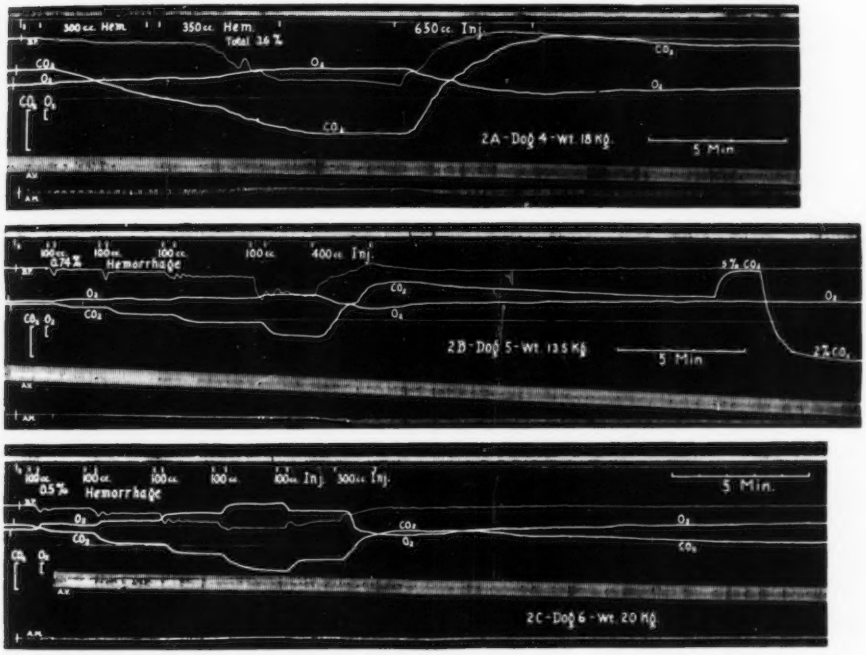

Fig. 2

Again the effect is largest with the last hemorrhage. It should be added, however, that hemorrhages of this extent do not always show impaired expiratory exchange under the conditions of our experiments. Occasionally vigorous animals fail to show the usual response in the early stages of hemorrhage. On the other hand, decidedly smaller hemorrhages than 0.50 per cent of the body weight produced demonstrable changes in the expired gases. Herein lies a distinct advantage of the continuous method of recording gaseous exchange. Changes which are undetectable by gaseous analysis are positively recorded by permanent breaks in the curve.

The records bring out another point which is worthy of comment, namely, the effectiveness of intravenous injection of saline solution on gaseous exchange. The improvement of expiratory exchange was pointed out in figure 3B, in which the blood volume was augmented by injection of 250 cc. of saline solution. A second improvement is seen on the following injection of 450 cc. of saline solution. The effectiveness of saline solution comes out still more clearly in figure 3C, in which striking carbon dioxide overshooting and oxygen over-retention develop when saline solution is substituted for reinjection of blood. Obviously the acceleration of a simple circulating fluid such as plasma or saline solution should improve gaseous transport. On the other hand, the improvement which obtains seems out of proportion to such possibilities and supports the view of improved gaseous metabolism by virtue of an augmented nutrient flow (concentration of blood times volume-flow) (Gesell, 1919).

In analyzing the respiratory response to changes in blood volume, as indicated by the abdominal movements, the behavior of the animal may be divided into two main types. In the first type (figs. 3A, 3B, 1A and 1B) hemorrhage elicits stimulation of respiratory movements and injection an immediate progressive decrease. In the second type, hemorrhage first elicits stimulation, which gives way to depression of varying degrees towards the close of hemorrhage. Injection then increases respiratory movements which soon subside (see figs. 1C, 2A, 2B, 2C and 3C). The depressing effects of hemorrhage as well as the stimulating effects, and the augmenting as well as the quieting effects of injection are thus illustrated. For purposes of generalization, barring the intermediate effects of depression at the close of hemorrhage and augmentation at the beginning of injection in the second type of response, all of the records show stimulation with decreased blood volume and decreasing respiratory movements with increased blood volume. This leaves, for the present, the intermediate period unexplained.

The behavior in general, however, might conceivably be explained on the basis of changes in rate of oxidation, for an inverse relation of these functions seems to hold in a gross way though it must be admitted that a more careful analysis leads to difficulty even in the type 1 response. For example (see figs. 3A and B), it is fair to assume that oxidations are greatest immediately after reinjection from which they taper off to normal. However, during this particular period respiratory movements diminish as oxidations diminish. Yet comparing respiratory movements during the period of depleted blood volume with those at the end of recovery the inverse relation holds. Obviously if rate of oxidations is significant there are other predominating influences. We, therefore, prefer to analyze the respiratory movements from the standpoint of an acid mechanism of control as well.

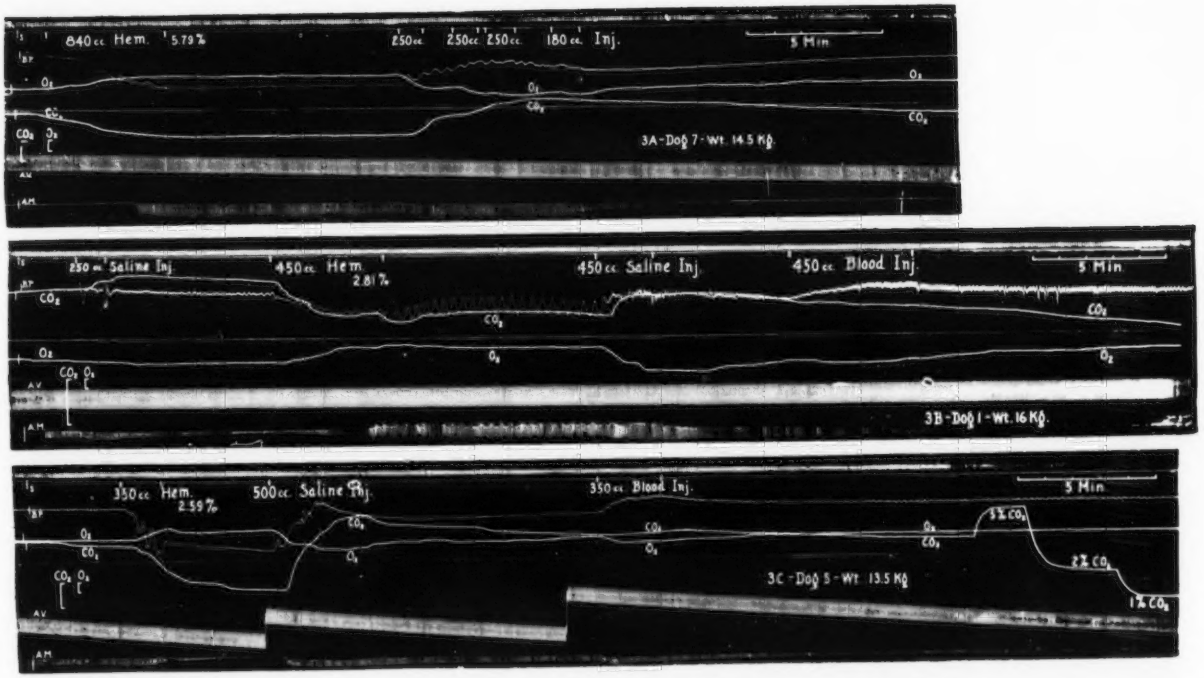
840 cc. Hem. 5.79%
250 cc. 250 cc. 250 cc. 180 cc. Inj.
5 Min
3A-Dog 7-Wt. 14.5 Kg.
250 cc. Saline Inj.
450 cc. Hem. 2.81%
450 cc. Saline Inj.
450 cc. Blood Inj.
5 Min
3B-Dog 1-Wt. 16 Kg.
350 cc. Hem. 2.59%
500 cc. Saline Inj.
350 cc. Blood Inj.
5 Min
5% CO2
2% CO2
1% CO2
3C-Dog 5-Wt. 13.5 Kg

Fig. 3

With reduced elimination of carbon dioxide during hemorrhage acid will accumulate if production continues at the usual rate. Also, an acid effect may result though the total production of acid be reduced, provided an increasing proportion of this acid is fixed (lactic acid). It is not an unreasonable assumption that anaerobic products of metabolism accumulate during the period of depleted blood volume. Granting that the concentration of anaerobic metabolites within the cell during hemorrhage is a contributory factor establishing the high rate of oxygen consumption on reinjection, then our records indicate that concentration has occurred. Further contributory evidence towards the theory of an acid mechanism of control is, therefore, furnished by these experiments. The experiments on the acidity of the circulating blood (Hertzman and Gesell, 1927), also support this view. The effects of hemorrhage and injection on blood acidity are recalled. Though pulmonary ventilation is maintained at a constant volume, hemorrhage invariably produced increased alkalinity of the arterial blood, showing that the more slowly the blood flows through the lungs the better it is ventilated. This highly alkalinized blood (more alkaline than normal) is similarly retarded in the tissues and by the time it has arrived at the veins it is more acid than normal venous blood, which demonstrates the acidifying effects of a retarded circulation and offers fairly direct proof of increasing acidity of the tissues during hemorrhage. On reinjection of the blood the arterial blood swings acid and the venous as a rule swings alkaline. The acid swing is sharp and excessive, shortly giving way towards normal values. This acid overshooting of the arterial blood must be related to the carbon dioxide overshooting in the expired gas experiments. It is interesting that the venous blood during this period of overshooting may actually turn alkaline as it is carrying increased amounts of carbon dioxide to the lungs. Acid overshooting at the venous side, however, may occur as well as on the arterial side. The acid values of the arterial and venous blood during the recovery period after reinjection may remain considerably above the normal acid values before hemorrhage. This was taken as supporting evidence that lactic acid, formed in greater abundance during depleted blood volume, had left the tissues in greater amount and reduced the buffer base of the blood. On the whole it can be said that the findings of the blood acidity experiments during hemorrhage agree with those of expired gases. The direct experiments of Riegel (1927) show that lactic acid accumulates in the blood during hemorrhage. The gradual augmentation of ventilation during hemorrhage and gradual recovery during reinjection seem to correspond with a gradual increasing and decreasing acidity of the tissues respectively.

To be sure the results illustrated in the response of type II offer exceptions in the intermediate regions previously referred to. Continued hemorrhage may lead to decreased ventilation and subsequent reinjection to

temporary increased ventilation. It is convenient to think of the depressing effect of lack of oxygen and the reviving effect of replenishment but in so doing we are mindful of the meaninglessness of these terms. Until we can think of this altered behavior in more specific terms we must admit that a final explanation is not at hand. It is conceivable, of course, that the energy for the rhythmic process is no longer sufficiently available with prolonged or severe disturbances in oxidation, and that reinjection provides this deficiency. Granting this, the acid mechanism may be found to be a predominating factor.

SUMMARY AND CONCLUSIONS

The effects of hemorrhage and injection on expired carbon dioxide and expired oxygen were studied with continuous electrometric methods.

Constant artificial ventilation with room air was administered during pneumothorax.

Hemorrhage was found to elicit a prompt decrease in expired carbon dioxide and retained oxygen. On reinjection carbon dioxide elimination momentarily increased above the pre-hemorrhage elimination to return approximately to the pre-hemorrhage level. This period of carbon dioxide overshooting was accompanied by a corresponding over-oxygen retention which showed similar recovery to normal values.

Injection of isotonic saline solution following hemorrhage elicited similar changes in expired gases as did the reinjection of blood.

In general, hemorrhage elicited increased respiratory movements and reinjection decreased movements.

Though the susceptibility to hemorrhage varied with the animal a gross relation between reduction in blood volume and impairment of gaseous exchange was established by comparing effects produced by varying hemorrhages in different animals.

In a limited number of experiments on the effects of graded hemorrhage on the same animal it was found that the later the hemorrhage in the series the greater was the impairment of gaseous exchange per unit of hemorrhage.

Records are shown in which an initial hemorrhage of 0.50 per cent of the body weight produced appreciable changes in the expired gases. Although in some animals such hemorrhage may elicit no demonstrable effects, in others a decidedly smaller hemorrhage produces unmistakable effects.

The effects of hemorrhage and reinjection on expiratory exchange were attributed to changes in gaseous transport associated with changes in the volume-flow of blood. The improvement of gaseous exchange resulting from the injection of isotonic salt solution was attributed to augumented nutrient flow.

The decreased oxygen retention and decreased carbon dioxide elimination during the period of depleted blood volume suggested increased anaero-

bic acid formation and accumulated acid retention in the body. The hyperoxygen retention and carbon dioxide elimination on reinjection indicated increased removal of accumulated anaerobic metabolites.

The changes in respiratory movements associated with hemorrhage and reinjection are discussed in relation to an acid mechanism of respiratory control.

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THE REGULATION OF RESPIRATION

XV. Effects of Intravenous Injection of Sodium Bicarbonate and Sodium Carbonate on Expired Carbon Dioxide and Expired Oxygen as Studied with Continuous Electrometric Method[1]

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It is a well-known fact that intravenous injection of sodium bicarbonate and sodium carbonate elicit opposite responses of the circulatory and respiratory systems (Scott 1918; Dale and Evans, 1922; Collip, 1920-21; Gesell, 1923a, b; 1925, 1926 and others). Sodium carbonate almost invariably produces a fall in mean blood pressure and a supression of respiratory movements, while sodium bicarbonate brings on a rise in mean blood pressure and increased respiratory movements. Inasmuch as both of these salts increase the alkalinity of the blood the findings were contrary to the prevalent theories on the dependency of the activity of the bulbar centers on the acidity of the arterial blood, and the failure to link the behavior of these centers to associated changes in acidity led to explanations based on the specific effects of these salts and carbon dioxide.

Assuming that acidity of the blood is of relatively little significance in the control of ventilation one of us (Gesell 1923a, 1925) attempted to relate the behavior of the respiratory center to changes in acidity of the interior of the cell associated with injection of sodium carbonate and sodium bicarbonate. Granting a relative impermeability of the cell membrane to the metallic cation, a change in the hydrogen ion concentration of the cell may be limited primarily to the other constituents of a carbonate buffer system. If the movement of acid between the cell and blood is determined by the relative pressures of CO_2, H_2CO_3, $H' \cdot HCO_3$ and HCO_3' ions within and without the cell a mechanism appears to exist which would increase the acidity of the interior of the cell on administration of sodium bicarbonate and decrease it on the administration of sodium carbonate.

By means of a simple diffusion experiment without the aid of a membrane and with the use of indicators the diffusion of hydrochloric acid from a point of lower concentration (but high concentration of sodium chloride) to a point of higher concentration was demonstrated to occur within a

[1] Preliminary report in This Journal Proc. Amer. Physiol. Soc., 1927, lxxxi, 495.

few seconds (Gesell, 1923b). Such experiments indicate a possible effect on the acidity of the cell of increasing the concentration of the HCO_3' anion of the circulating blood. But the effects of sodium bicarbonate are not so simple, for dissolved CO_2 and undissociated H_2CO_3 are increased as well. The free movement of these two constituents through cell membranes provides another mechanism of acid accumulation within the cell. This reasoning has been supported in in-vitro experiments on carbonate buffer systems (Gesell, 1923b), and has been upheld in the in-vivo experiments on the dog (Gesell and Hertzman, 1926). Whereas sodium carbonate was found to increase the alkalinity of the cerebrospinal fluid and reduce pulmonary ventilation, sodium bicarbonate was found to increase the acidity of the fluid and stimulate respiratory movements.

The object of these experiments is to gain information on the elimination of carbon dioxide with the administration of sodium bicarbonate and carbonate. Since the blood may be considered as lying between the tissues and the alveolar air it was assumed that if these salts influence the movement of acid between the blood and the tissues they will by the same mechanism influence the movement between the blood and the alveolar air. By using our continuous methods of recording expired carbon dioxide and expired oxygen such changes in movement of carbon dioxide should be easily followed.

Method. Artificial ventilation of constant volume was administered during pneumo-thorax as in the preceding experiments. Molecular sodium bicarbonate and half molecular carbonate in amount and rate indicated in the figures were injected intravenously by way of the femoral vein. As a further guide to the size of injection the weight of the dog is marked on the record. In general the description of the figures given in the first of the expired gas experiments sufficies for these experiments. Figures 3A, B, C, D, E, F and G and figures 4A and B are supplemented by expired carbon dioxide curves established by gasometric analysis with the Haldane-Henderson gas analyzer (broken line curves). The periods of obtaining the gas samples are marked by horizontal bars on the signal line. The "expiratory quotients" are given as well.

Results. The results described are found in figures 1 to 4 selected from 14 experiments of a total of 26, all of which were performed on dogs under morphine urethane anesthesia.

A cursory inspection of the figures will show that the intravenous injection of sodium bicarbonate increased the expired carbon dioxide (see figs. 1A, B and C, 2A, B, C, E, G and I, 3A, B, C, D, F and G and 4A and B), and that the intravenous injection of sodium carbonate produced the opposite effect (see figs. 1A, B and C, 2D, F, H and I, 3E and 4A and B). Similarly, reviewing the effects of these salts on mean blood pressure and abdominal movements it will be seen that sodium bicarbonate increased the

mean blood pressure and abdominal respiratory movements, and that sodium carbonate in general elicited a fall in mean blood pressure and a reduction of respiratory movements.

Without further analysis it might be assumed that our results confirm the view that the addition of sodium carbonate to the blood lowers the carbon dioxide pressure of the blood, thereby leading to a greater movement of carbon dioxide from the tissues into the blood and a reversal of the usual movement between the alveolar air and the circulating blood, that the addition of sodium bicarbonate accelerates the movement of carbon dioxide from the blood into the alveolar air and retards or reverses the usual movement of carbon dioxide from the tissues into the circulating blood. Be this as it may, the oxygen records indicate that there is a modifying factor influencing expiratory exchange, for injection of either salt led to an increased oxygen retention. The effects of bicarbonate and carbonate on oxygen consumption may be compared to best advantage in the experiments in which both salts were injected in the same animal (see figs. 1A, B and C, 2B and C, 2E and F, 2G and H, 2I, 3B and E, 4A and 4B). That the increased oxygen retention is not a result of a transitory increased saturation of hemoglobin alone is clearly indicated in figures 2A and 2B, where injection is continuously maintained over a long period of time. Oxidations in the tissues are unquestionably augmented. Obviously, increased oxidations must tend towards greater elimination of carbon dioxide. Thus in the case of sodium carbonate retention of carbon dioxide by the blood becomes less obvious and in the case of sodium bicarbonate the augmented elimination of carbon dioxide more pronounced. Despite this fact sodium carbonate is seen to lead to marked and prolonged retention of carbon dioxide. See figures 1A and B, 2F, H and I. Exceptions to prolonged retention of carbon dioxide are seen in figures 3E, and 4A, and 1C.

Reinspection of the records shows at once that the increased oxidations cannot account entirely for the increased elimination of carbon dioxide with administration of sodium bicarbonate, for the changes in expired carbon dioxide are considerably greater than the corresponding changes in expired oxygen. To be more exact on this point we collected gas samples at selected points and analyzed them for changes in expired oxygen and carbon dioxide with the Haldane-Henderson gas analyzer. The experiments in which such analyses were made are illustrated in figures 3A, B, D, E, F and G and 4A and B. The curves of expired carbon dioxide, established by the gas analyses are shown in broken line to distinguish them from the continuously recorded curves. By dividing the expired carbon dioxide by retained oxygen[2] (20.94 minus the expired oxygen in

[2] The usual corrections made in the accurate determinations of respiratory quotients were not made. For convenience we shall refer to the value 20.94 minus expired oxygen as "oxygen retained."

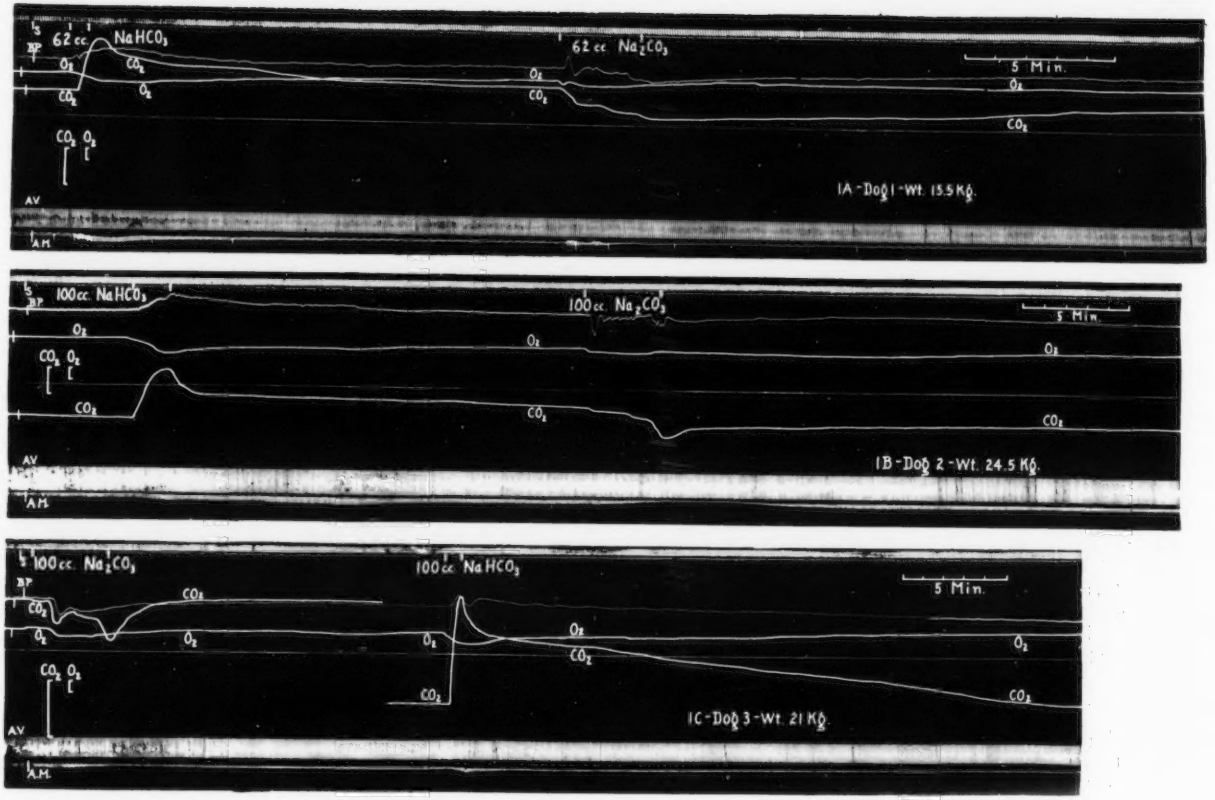
62 cc. NaHCO3
62 cc. Na2CO3
5 Min.
1A-Dog 1-Wt. 15.5 Kg.
100 cc. NaHCO3
100 cc. Na2CO3
5 Min.
1B-Dog 2-Wt. 24.5 Kg.
100 cc. Na2CO3
100 cc. NaHCO3
5 Min.
1C-Dog 3-Wt. 21 Kg.
AV
A.M.

Fig. 1

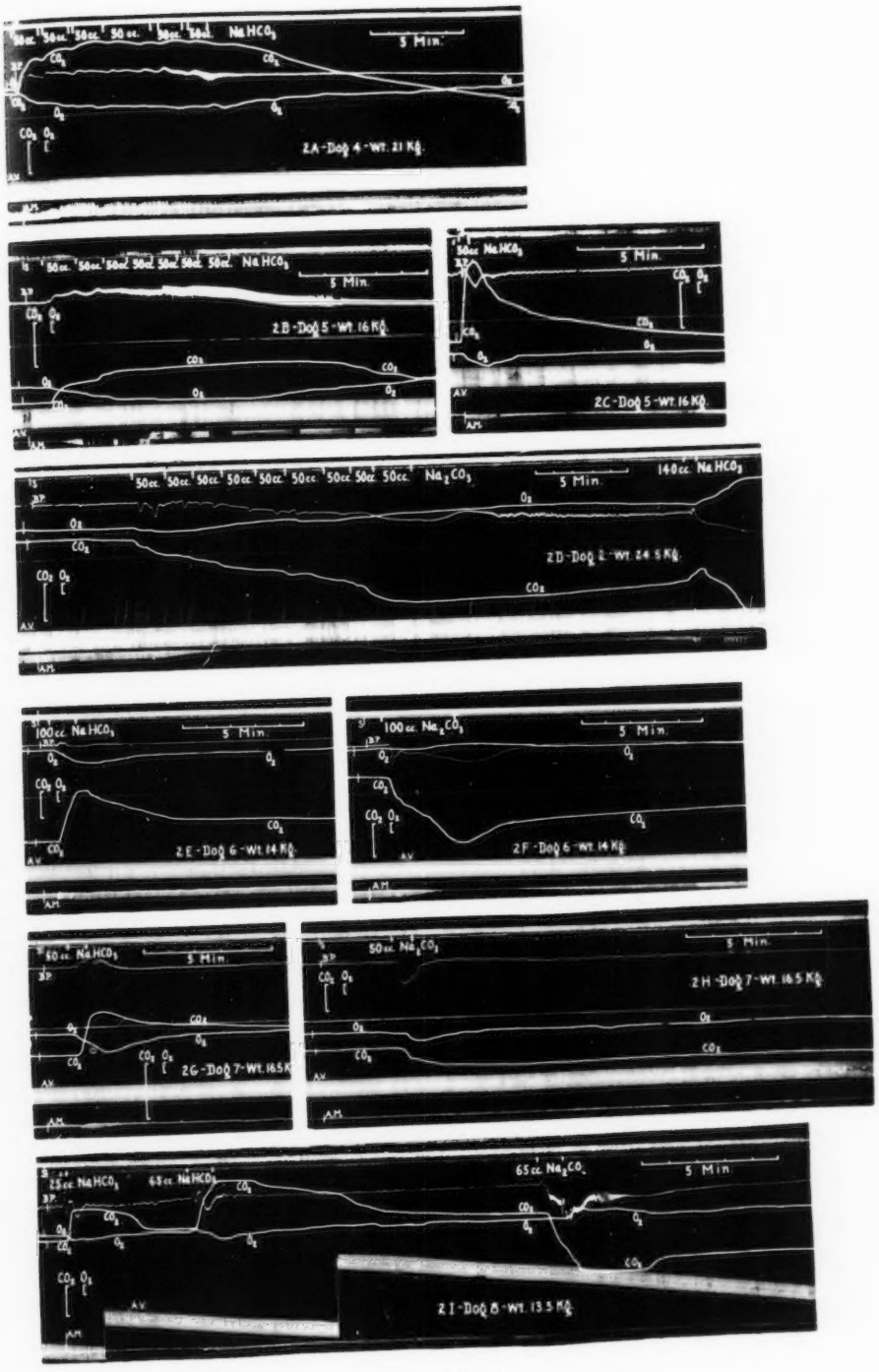
50cc. 50cc. 50cc. 50cc. 50cc. 50cc. NaHCO3
5 Min.
2A - Dog 4 - Wt. 21 Kg.
50cc. 50cc. 50cc. 50cc. 50cc. 50cc. 50cc. NaHCO3
5 Min.
2B - Dog 5 - Wt. 16 Kg.
50cc. NaHCO3
5 Min.
2C - Dog 5 - Wt. 16 Kg.
50cc. 50cc. 50cc. 50cc. 50cc. 50cc. 50cc. 50cc. Na2CO3
5 Min.
140cc. NaHCO3
2D - Dog 2 - Wt. 24.5 Kg.
100cc. NaHCO3
5 Min.
2E - Dog 6 - Wt. 14 Kg.
100cc. Na2CO3
5 Min.
2F - Dog 6 - Wt. 14 Kg.
50cc. NaHCO3
5 Min.
2G - Dog 7 - Wt. 16.5 Kg.
50cc. Na2CO3
5 Min.
2H - Dog 7 - Wt. 16.5 Kg.
25cc. NaHCO3
65cc. Na2CO3
5 Min.
2I - Dog 8 - Wt. 13.5 Kg.

Fig. 2

TABLE 1

EXPERIMENT NUMBER	SAMPLE NUMBER	EXPIRED O_2	RETAINED O_2	EXPIRED CO_2	EXPIRATORY QUOTIENT	MAXIMUM INCREASE OF CO_2 OVER PRE-INJECTION LEVEL	REMARKS
		per cent	*per cent*	*per cent*		*per cent*	
3A	1	16.17	4.77	4.34	0.91		
	2	14.56	6.38	9.10	1.42	109	100 cc. M/1 $NaHCO_3$
	3	13.93	7.01	7.51	1.07		
	4	14.30	6.64	6.98	1.05		
	5	14.95	5.99	5.95	0.99		
	6	15.61	5.33	5.10	0.96		*
3B	1	18.18	2.76	2.62	0.95		100 cc. M/1 $NaHCO_3$
	2	17.88	3.06	4.74	1.55	84	
	3	17.01	3.93	5.92	1.50		
	4	16.65	4.29	4.90	1.14		
	5	17.76	3.18	3.61	1.13		
	6	17.90	3.04	3.22	1.06		
	7	17.85	3.09	2.88	0.93		*
	8	17.96	2.98	2.76	0.92		*
3D	1	16.98	3.96	3.44	0.87		
	2	15.94	5.00	5.72	1.14	66	52 cc. M/1 $NaHCO_3$
	3	16.91	4.03	4.30	1.07		
	4	16.91	4.03	3.87	0.96		
3E	1	17.96	2.98	2.76	0.92		
	2	17.53	3.41	2.23	0.65		100 cc. M/2 Na_2CO_3
	3	17.62	3.32	1.73	0.52		
	4	17.58	3.36	2.75	0.82		
	5	17.54	3.40	2.91	0.86		
	6	17.64	3.30	2.88	0.87		
3F	1	16.50	4.44	5.49	1.24		†
	2	14.82	6.12	7.22	1.18	31	52 cc. M/1 $NaHCO_3$
	3	16.25	4.69	6.36	1.35		*
	4	16.58	4.36	5.70	1.31		
3G	1	16.99	3.95	3.21	0.81		
	2	16.71	4.23	3.45	0.82	7	100 cc. M/1 NaCl
	3	16.91	4.03	3.31	0.82		
	4	16.97	3.97	3.22	0.81		
	5	15.56	5.38	5.25	0.98	63	100 cc. M/1 $NaHCO_3$
	6	16.68	4.26	4.37	1.02		
	7	16.84	4.10	3.50	0.85		
	8	16.77	4.17	3.48	0.83		
	9	16.63	4.31	3.51	0.81		*

* Not shown on the record.

† 3 per cent CO_2 in room air administered during this experiment.

TABLE 1—*Concluded*

EXPERIMENT NUMBER	SAMPLE NUMBER	EXPIRED O_2	RETAINED O_2	EXPIRED CO_2	EXPIRATORY QUOTIENT	MAXIMUM INCREASE OF CO_2 OVER PREINJECTION LEVEL	REMARKS
		per cent	*per cent*	*per cent*		*per cent*	
4A	1	15.56	5.38	4.38	0.81		100 cc. M/1 NaCl
	2	15.17	5.77	4.76	0.83	8	
	3	15.07	5.87	4.68	0.80		100 cc. M/1 $NaHCO_3$
	4	13.82	7.12	7.74	1.09	67	
	5	14.00	6.94	6.51	0.94		
	6	14.38	6.56	5.40	0.82		
	7	14.04	6.90	4.05	0.59		100 cc. M/2 Na_2CO_3
	8	14.47	6.47	5.16	0.80		
	9	14.27	6.67	5.44	0.82		100 cc. M/1 NaCl
	10	13.90	7.04	5.97	0.85	9	
	11	13.92	7.02	6.26	0.89		
4B	1	16.63	4.31	3.51	0.81		100 cc. M/2 Na_2CO_3
	2	16.11	4.83	3.11	0.64		
	3	16.63	4.31	3.46	0.80		
	4	16.37	4.57	3.79	0.83		
	5	16.32	4.62	4.07	0.88	7	100 cc. M/1 NaCl
	6	16.44	4.50	3.90	0.87		
	7	15.90	5.04	5.06	1.00	29	100 cc. M/1 $NaHCO_3$
	8	16.41	4.53	4.25	0.94		

percentage) we have arrived at a helpful figure which we designate as the "expiratory quotient," rather than respiratory quotient. The value of these quotients is designated on the records at each point of gas sampling. The figures from which these quotients are computed appear in table 1, which shows, in addition, the maximum percentage increase in expired carbon dioxide above the preinjection value.

Inspection of table 1 shows enormous changes in the elimination of carbon dixoide. The first observation, figure 3A, from 4.34 per cent to 9.10 per cent or an increase of 109 per cent, the second observation, figure 3B, from 2.62 per cent to 4.74 per cent or an increase of 84 per cent, and the third observation, figure 3D, from 3.44 per cent to 5.72 per cent or an increase of 66 per cent. Comparing the changes in expiratory quotients in the same observations—in number one it is increased from 0.91 to 1.42, in number two from 0.95 to 1.55 and in number three from 0.87 to 1.14.

From the shape of the expired carbon dioxide curves it must be obvious that the magnitude of the maximum expiratory quotient which is obtained is a matter of chance, as the period of very rapid elimination is only a matter of a few seconds. This highly augmented and transitory elimination

of carbon dioxide we consider one of the striking points of this paper. In some instances it is so short (figs. 3A and 3B) that the carbon dioxide electrode fails to come into equilibrium rapidly enough to register the peak of the change. In figures 1C, 2C and 3C the electrode appears to catch the major changes and give a fairly complete picture of carbon dioxide elimination. Gas samples taken five seconds too early or too late will, therefore, give decidedly low, maximum expiratory quotients. This may account in part for the variations in the quotients given in table 1.

In using the expiratory quotient in the interpretation of our results in this and following papers we do not imply that it invariably approximates the true respiratory quotient, but believe that the method employed of eliminating variations in pulmonary ventilation gives greater significance to the ratio of expired carbon dioxide to oxygen retained, for it eliminates the washing out effect of augmented ventilation.

In these particular experiments the marked suddenness of onset and the short duration of the greatly increased expiratory quotient have special interest. They are not in disagreement with a short transitory salt effect upon the blood expelling carbon dioxide. But other contributing effects should also be considered. Of these we are reminded of the effects which changes in volume-flow of blood may have upon the amount of carbon dioxide washed from the blood per unit of time as it circulates through the lungs. Since increased pulmonary ventilation leads to a better elimination of carbon dioxide from the blood with a resulting increase in the expiratory quotient it seems reasonable to expect that a greater flow of blood, even though pulmonary ventilation remains constant, should lead to increased carbon dioxide elimination in the absence of changes in gaseous metabolism at the tissues. The augmenting effects of sodium bicarbonate and carbonate on the volume-flow of blood (Bronk and Gesell, 1927), therefore, suggest a second contributing factor to the increased expiratory quotient. We are not prepared to say how significant this factor is but have found that injection of molecular solution of sodium chloride, which undoubtedly leads to increased flow of blood, produced relatively small changes in expired carbon dioxide and expiratory quotient as compared with those produced by injection of sodium bicarbonate (see figs. 3G, 4A and B and table 1). In figure 3G the effects of injection of 100 cc. molecular sodium chloride and 100 cc. molecular sodium bicarbonate are compared in the same animal. The expired carbon dioxide is increased from 3.21 per cent to only 3.45 per cent. The expired oxygen is diminished a corresponding amount. The expiratory quotient remains virtually unchanged. Figure 4A shows a more complete experiment. Sodium chloride is injected twice, once at the beginning and again at the end. The drift of the carbon dioxide electrode conceals the increased elimination of carbon dioxide in the first record which appears in the gasometric

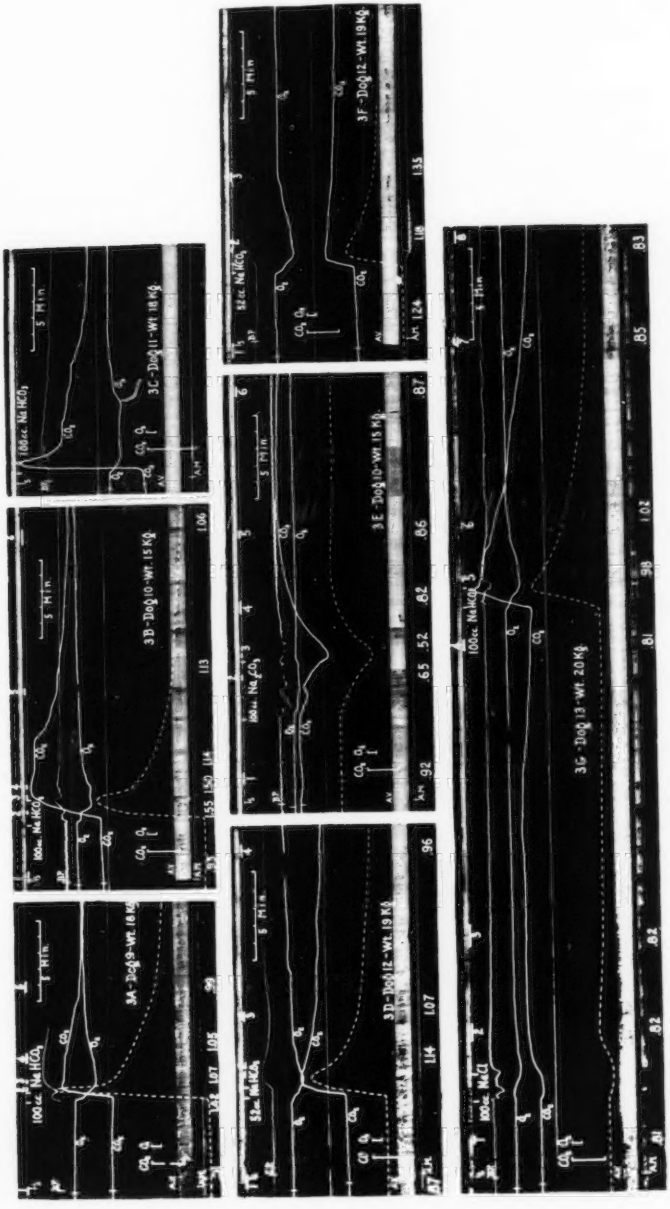
100cc. NaHCO3
5 Min.
3A - Dog 9 - Wt. 18 Kg.
100cc. NaHCO3
3B - Dog 10 - Wt. 15 Kg.
100cc. NaHCO3
3C - Dog 11 - Wt. 18 Kg.
50cc. NaHCO3
3D - Dog 12 - Wt. 19 Kg.
100cc. Na2CO3
3E - Dog 10 - Wt. 15 Kg.
50cc. NaHCO3
3F - Dog 12 - Wt. 19 Kg.
100cc. NaCl
3G - Dog 13 - Wt. 20 Kg.

FIG. 3

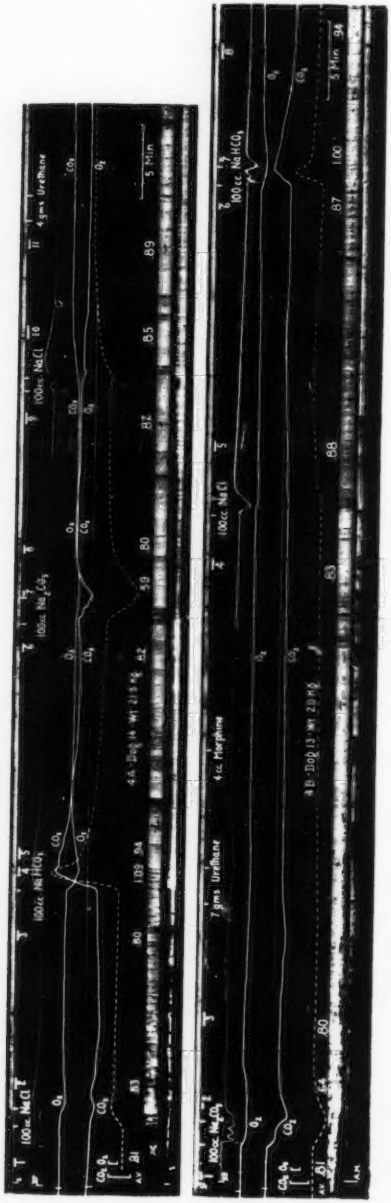

Fig. 4

analysis. Whereas the expired carbon dioxide increases only 0.38 per cent and 0.53 per cent in the first and second administration of sodium chloride with respective increases in expiratory quotients of 0.02 and 0.03 sodium bicarbonate increased the expired carbon dioxide 3.06 per cent and the expiratory quotient 0.31. In figure 4B sodium chloride increased the expiratory quotient 0.05 and sodium bicarbonate increased it 0.13. The results indicate that changes in volume-flow of blood though not exerting a predominant effect on the expiratory quotient constitutes a contributory factor.

As another contributing factor we turn to the experiments of Macleod and Hoover (1916) and Macleod and Knapp (1918) showing accumulation of lactic acid in the blood on the administration of sodium bicarbonate. Presumably the appearance of lactic acid in the blood results in displacement and increased elimination of carbonic acid. But as no lactic acid determinations were made in our experiments we prefer to postpone an evaluation of the various factors influencing the expiratory quotient on the administration of sodium bicarbonate for a future paper. For the present we believe that a number of factors are at work producing an increased elimination of carbon dioxide and an increased expiratory quotient on the administration of sodium bicarbonate.

We have barely discussed the results on the administration of sodium carbonate except in comparing the effects with those of sodium bicarbonate on rate of oxidation and on respiratory movements, and of its effects upon retention of carbon dioxide. In this latter connection it is not amiss to call attention to the reduction in the expiratory quotient produced by sodium carbonate (see figs. 3E, 3G, 4A and 4B and table 1).

Discussion. If it is correct that intravenous injection of sodium carbonate decreases the acidity of the interior of the cell and that sodium bicarbonate increases it we are led to interesting speculation. Knowing that both salts exert a distinct alkaline effect on the blood, and assuming a free movement of base through the salt-permeable capillaries it is possible (though not proved) that both salts exert an alkaline effect upon the immediate environment of the cells of the body including the respiratory neurones. With such conditions obtaining the predominating effect of changes in acidity of the interior of the cell on this rhythmic discharge is suggested.

Considering these acid relations in and outside of the cell with respect to oxidations the significance of the reaction of immediate environment rather than the cell interior is suggested, for sodium bicarbonate and sodium carbonate both increased oxidations, though theoretically they may produce opposite effects on the acidity of the interior of the cell.

In this connection exception to the inverse relation of rate of oxidation to pulmonary ventilation is pointed out again. Though increased oxida-

tions are elicited by both sodium carbonate and bicarbonate, pulmonary ventilation is depressed only on the administration of sodium carbonate. The ventilatory response appears to conform rather to an acid mechanism of control. As further evidence attention is here called to figure 2D in which a large amount of sodium carbonate was administered over a prolonged period. The first effect is increased rate of oxidation accompanied by decreased ventilation. But with further injection when oxidations begin to diminish considerably below the preinjection level pulmonary ventilation continues depressed. Later during recovery, as oxidations increase ventilation is also increased.

We call attention to the fact that though sodium bicarbonate and sodium carbonate, which theoretically may produce opposite changes in acidity of the interior of the cell, produce the same augmentation of oxidations. It is possible that oxidations are increased by increased alkalinity of the immediate cell environment, which may be common to the administration of both salts. It does not appear likely that the large increase in oxygen consumption, noted for example in figures 1A and 1B, is due to increase in volume-flow of blood. This is indicated in figures 4A and B, where the effects of intravenous injection of molecular sodium chloride may be compared with the effects of sodium bicarbonate and carbonate.

SUMMARY AND CONCLUSIONS

The effects of intravenous injection of sodium carbonate and sodium bicarbonate on expired carbon dioxide and expired oxygen during constant artificial administration of room air were studied. Continuous electrometric methods checked by gas analyses were used.

Sodium carbonate elicited decreased elimination of carbon dioxide and increased retention of oxygen, which was followed by a gradual recovery.

Sodium bicarbonate elicited an increased elimination of carbon dioxide and increased retention of oxygen followed by a gradual recovery. For a period of a few seconds the elimination of carbon dioxide was enormously augmented.

The increased elimination of carbon dioxide produced by sodium bicarbonate was considerably in excess of the increased oxygen retention.

The "*expiratory quotient*" $\frac{\text{Expired } CO_2}{\text{Retained } O_2}$ obtained during constant pulmonary ventilation was used as a guide to interpret the expiratory exchange.

In one experiment the expired carbon dioxide was increased from 4.34 per cent to 9.10 per cent, a total increase of 109 per cent. The associated change in expired oxygen was from 16.17 per cent to 14.56 per cent. These figures correspond to an increase in the expiratory quotient from 0.91 to 1.42.

Three contributing factors towards this high expiratory quotient were considered: 1, the direct salt effect of $NaHCO_3$ on the carbonate buffer system of the blood tending to drive carbonic acid from the blood in two directions—into the tissues and into the alveolar air; 2, the increased ventilation of the blood resulting from increased volume-flow of blood; 3, the liberation of carbon dioxide from the buffer base of the blood by increased diffusion of lactic acid from the tissues.

Though a quantitative evaluation of the part played by each of these factors on the magnitude of the expiratory quotient following the administration of sodium bicarbonate cannot at present be made it was suggested that the first factor is accountable for the difference in effects of sodium carbonate and sodium bicaronbate on ventilation and circulation.

The results on expiratory exchange support the view that whereas sodium carbonate increases the alkalinity of the cells, sodium bicarbonate may temporarily increase the acidity. On that basis the respiratory movements associated with the administration of these salts may be explainable by an acid mechanism.

The fact that both salts increase the rate of oxidation but produce opposite effects on respiratory movements supports the results of the two preceding papers—that rate of oxidation is not a determining factor in respiratory control.

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THE REGULATION OF RESPIRATION

XVI. The Effects of Intravenous Injection of Sodium Cyanide on Gaseous Exchange and Acid Metabolism[1]

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In another paper the results of administration of gases of low oxygen tension on the composition of the expired gases (Gesell and McGinty, p. 323) and on the acidity of arterial and venous blood (Gesell and Hertzman, to be published) are given. It was pointed out that the function of the respiratory mechanism depends on the composition of the inspired gases, the rate and efficiency of the transport of these gases to and from the tissues and on the nature of the metabolism within the tissues. In this paper we are dealing with an altered metabolism produced by sodium cyanide in which an attempt is made to evaluate the effects of changes in tissue metabolism and of transport of gases in the blood by following the composition of the expired gas.

Sodium cyanide administration may be compared in many ways with low oxygen administration. In both there exists impaired oxidation in the tissues, in the latter, due to low oxygen tension and in the former due to direct effects of cyanide on the tissues. In both there is a broken coördination of the dual function of hemoglobin; during low oxygen administration the hemoglobin of the arterial and venous blood remains in a more or less reduced state, interfering with the liberation of carbon dioxide in the lungs; during cyanide administration the hemoglobin remains more or less oxidized in both arterial and venous blood, interfering with the removal of carbon dioxide from the tissues and its subsequent expulsion at the lungs. Finally, the effects of low oxygen and sodium cyanide on the acidity of the blood itself may be compared. Both low oxygen and cyanide increase the alkalinity of the blood by virtue of the increased ventilation and washing out of carbon dioxide. But when the effects of low oxygen and cyanide are compared during constant artificial ventilation the changes in blood acidity during these procedures differ. Low oxygen at first increases the alkalinity of the blood, which may be followed by increasing acidity as fixed acids and carbon dioxide accumulate in the tissues and neutralize the alkaline effect of reduced hemoglobin. Cyanide, on the other hand,

[1] Preliminary report in This Journal, Proc. Amer. Physiol. Soc., 1927, lxxxi, 495.

may lead at once to increasing acidity due to higher oxidation of the hemoglobin, which is augmented by progressive accumulation of acids in the blood. In both instances, however, respiratory activity is stimulated notwithstanding the different response in acidity of the blood, which seems to lend evidence to the importance of the metabolism of the respiratory cells themselves in the control of this activity. The changes in gaseous metabolism and transport described in this paper may, therefore, be compared with profit with similar changes produced by the administration of low oxygen.

METHOD. The procedure during these experiments is essentially the same as has been described in the preceding papers. Sodium cyanide in M/100 concentration was injected intravenously in the femoral vein in amounts indicated on the records during constant artificial respiration. Expired oxygen and carbon dioxide were followed by means of the continuous electrometric methods already described. In figures 3 and 4 expired gas samples were taken at designated intervals and analyzed gasometrically.

RESULTS. Records of 12 out of 19 experiments are presented. For a general description of these records see the preceding papers on expired gases in this number of THIS JOURNAL. In figures 3 and 4, in which expired gas samples were gasometrically analyzed the expiratory quotient is indicated below the corresponding gas sample. These figures are supplemented by table 1, giving the oxygen and carbon dioxide percentage of the expired air, the oxygen retained and the expiratory quotient.

An introductory review of the records shows in addition to a graded response to graded administration (see figs. 1E, F and G and 2A, B and C) four types of response. In the first type (see fig. 3C) sodium cyanide produces first a decrease in expired carbon dioxide and an increase in expired oxygen (or decrease in oxygen retained) followed, as the animal recovers, by increased carbon dioxide elimination and increased oxygen retention. This change continues until the expired carbon dioxide is considerably above the pre-injection level and until the oxygen retained is also greater than normal, when both again gradually return to approximately the pre-injection level. Other examples of this type of response in which there is a secondary overshooting of expired carbon dioxide and oxygen retained are found in figures 1A, 3B, 4A, 4B and 4C. A similar overshooting will be recalled in hemorrhage experiments and the experiments on the administration of low oxygen.

The second type of response is very similar to the first. The initial decrease in carbon dioxide elimination and oxygen retained and the secondary overshooting of carbon dioxide elimination occur. The difference between type I and II response lies in the absence of the secondary overshooting of retained oxygen. For examples see figures 1B–2B and 4A.

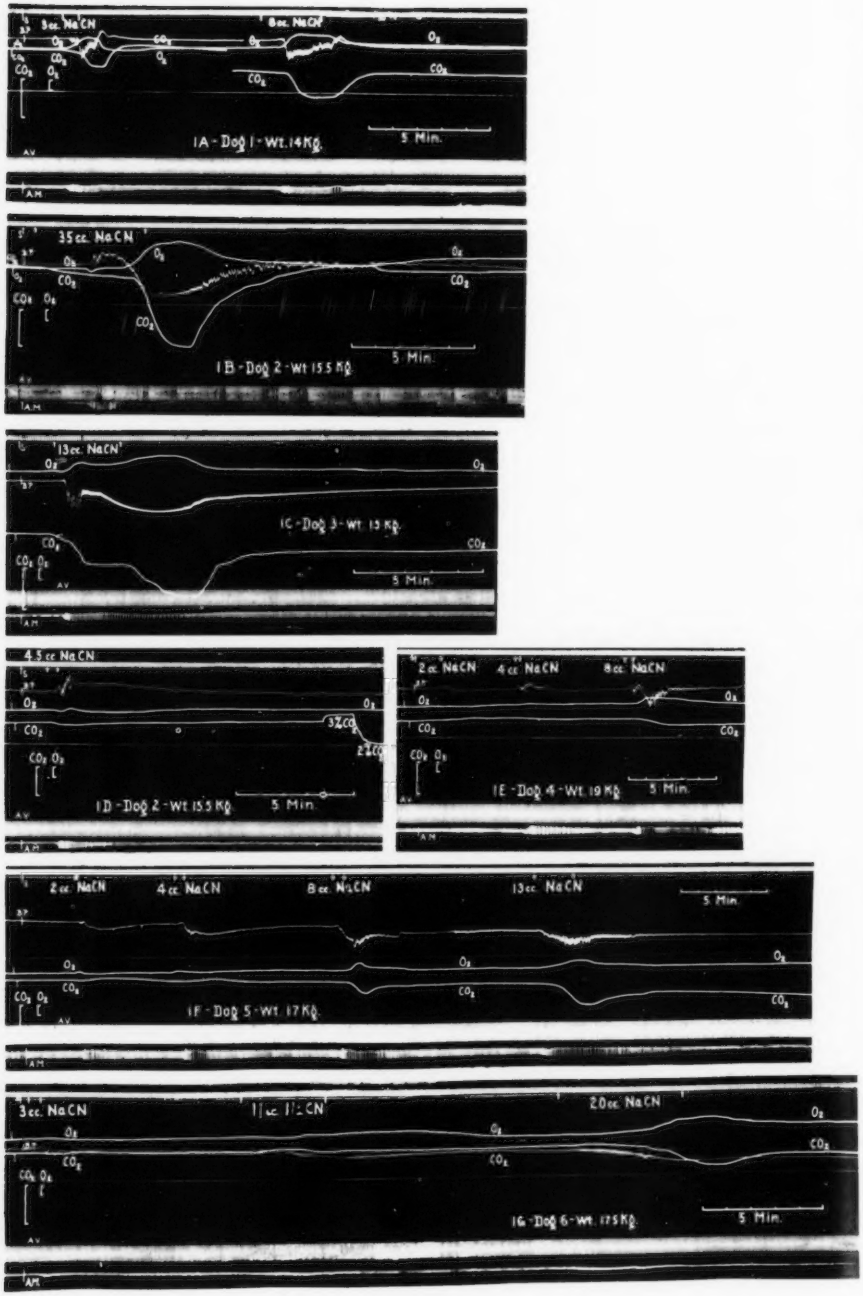
3cc. NaCN
8cc. NaCN
1A - Dog 1 - Wt. 14 Kg.
5 Min.
35cc. NaCN
1B - Dog 2 - Wt 15.5 Kg.
5 Min.
13cc. NaCN
1C - Dog 3 - Wt. 13 Kg.
5 Min.
4.5 cc NaCN
3%CO2
2%CO2
1D - Dog 2 - Wt 15.5 Kg.
5 Min.
2cc. NaCN
4cc. NaCN
8cc. NaCN
1E - Dog 4 - Wt. 19 Kg.
5 Min.
2cc. NaCN
4cc. NaCN
8cc. NaCN
13cc. NaCN
5 Min.
1F - Dog 5 - Wt. 17 Kg.
3cc. NaCN
11cc. NaCN
20cc. NaCN
1G - Dog 6 - Wt. 17.5 Kg.
5 Min.

Fig. 1

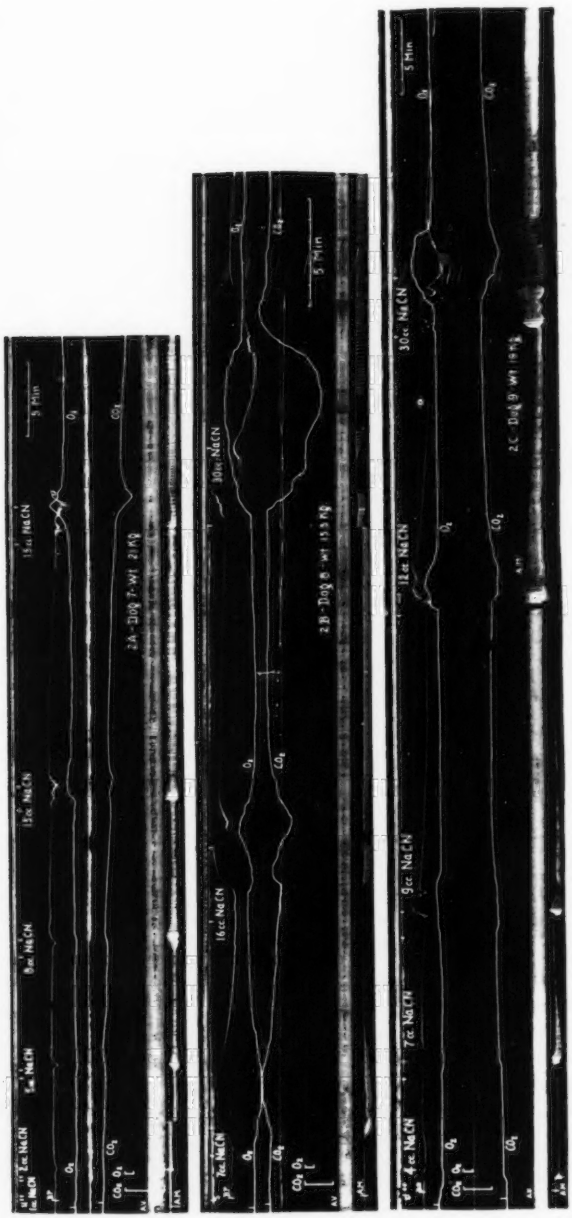

Fig. 2

In the third type of response the secondary overshooting of both expired carbon dioxide and retained oxygen are missing. Oxygen consumption and carbon dioxide elimination remain to varying degrees below the pre-administration values. For examples, see figures 1C, 1E, 1F and 1G. Analogous results with the administration of low oxygen to a point where recovery fails to occur are also recalled.

In the fourth type of response, which will be found in the experiments on the graded administration of cyanide there is a primary overshooting of expired carbon dioxide and retained oxygen, which may be followed by reverse changes and recovery. As the best examples see the first two injections of figure 2B. In the original records these same effects are distinctly apparent in the first injections of figures 2A, 2C, 1F and 1G and the first injection of 4B and 3B.

Type I, which is a common response to the administration of sodium cyanide provided the animal is in good condition and the injection is not too large, is similar to the usual response to hemorrhage and reinjection and to the administration of low oxygen and readministration of room air. It will be recalled that low oxygen decreased the carbon dioxide elimination and that on readministration of room air overshooting of expired carbon dioxide and retained oxygen occurred. These effects of low oxygen were attributed to impaired oxidations and impaired transport of acid from a broken coördination of the dual function of hemoglobin. The hemoglobin remains in a relatively constant reduced condition. On readministration of room air there is a compensatory increase in oxidations accompanied by improvement in the coördination of the dual function of hemoglobin. There is an increased oxidation and reduction of hemoglobin with each complete circulation of the blood. Comparable changes appear to explain the effects of cyanide. On the administration of sodium cyanide oxidations are impaired at the tissues. The oxyhemoglobin cannot be reduced as usual and on repassing through the lungs it becomes more completely oxidized. Less carbon dioxide is formed and its transport is impaired by the more constantly maintained state of oxidation of the hemoglobin. During the transitory state of impaired oxidations anaerobic products presumably increase and with recovery of oxidations lead to compensatory gaseous metabolism at the tissues. There is a consequent return of the normal cycle of reduction and of oxidation of the hemoglobin at the tissues and lungs respectively and a return of the coördinated function of hemoglobin. The gradual removal of the excess anaerobic products is the probable explanation of the return to more normal gaseous metabolism. This behavior of the animal to the administration of sodium cyanide is thus dependent on a reversibility of tissue metabolism.

In the low oxygen experiments we demonstrated the absence of overshooting of expired carbon dioxide and retained oxygen in those instances

in which impairment of oxidation was carried beyond the reversible stage. Where readministration of room air failed to produce the usual increased retention of oxygen, carbon dioxide overshooting failed to appear. It seems fairly safe to assume from the cyanide experiments (type III response), in which overshooting of expired carbon dioxide and retained oxygen are missing, that excess carbon dioxide elimination is associated with excess oxygen retention (1C, 1E, 1F, 1G and 2A). But that excess carbon dioxide elimination is not solely a matter of synchronous excess oxygen retention is indicated by the second type of response, in which overshooting of carbon dioxide elimination occurs in the absence of increased oxygen retention (see type II response 1B, 2B and 4A). Type II response may temporarily be looked upon as an intermediate stage between type I and III response. Justification of this classification is indicated by a more careful analysis of the gaseous metabolism.

In the experiments represented in figures 3 and 4 gasometric analyses of expired gas samples were made, the results of which are found on the records and in table I. Beginning again with our best example, figure 3C, we find that expired carbon dioxide is reduced from 3.51 per cent to 2.44 per cent and that retained oxygen is reduced from 3.78 per cent to 1.76 per cent. It will be noted that the change in retained oxygen is considerably greater than in expired carbon dioxide which results in an increase of the expiratory quotient from 0.93 to 1.39. From this point on up to sample 8 expired carbon dioxide increases to 4.20 per cent, which is considerably higher than the pre-administration level. Nevertheless the expiratory quotient falls from 1.39 to 0.87, accounted for by an associated increase in retained oxygen which is here 4.68 per cent as compared with 3.78 per cent before administration. From this point on the expiratory quotient continues to fall from 0.87 to 0.82 due to a disproportionate decrease in oxygen retained

Though several factors may be contributing to the changes in the expiratory quotient, it is believed that the changes in acid metabolism accompanying administration of and recovery from sodium cyanide are significant. When Hill (1922) and Meyerhoff (1921) (1922) published their quantitative data indicating the nature of the disappearance of lactic acid in muscle following contraction, one of us (Gesell, 1923a, 1925) arrived at conclusions which appear most important in explaining many respiratory phenomena and changes in the acid-base equilibrium of the body.

The production of lactic acid in resting muscle under anaerobic conditions and its removal in the presence of oxygen are well known facts. Thus for every four, five or six molecules of lactic acid depending upon the condition of the muscle only one molecule of acid is oxidized forming in the process three molecules of carbonic acid. The remaining lactic acid is transformed into its precursor.

From these figures we draw two conclusions relative to the total acidity of the muscle cell during anoxemia and subsequent replenishment of oxygen. For every four, five, or six molecules of lactic acid which fail to disappear as a result of anoxemia one, two or three extra molecules of acid will accumulate over and above the normal amount in the presence of oxygen. And conversely, for every four, five or six molecules of excess lactic acid which disappear on the replenishment of oxygen there will be a reduction in the total acidity represented by the disappearance of either one, two or three molecules of acid. (Gesell, 1923b.)

The effects of variations in oxygen supply were schematically represented as follows (Gesell, 1925):

$$(1)\ \frac{3\ H_2CO_3}{60\ NaHCO_3} \qquad (2)\ \frac{8\ H_2CO_3}{55\ NaHCO_3 + 5\ NaL} \qquad (3)\ \frac{3\ H_2CO_3}{60\ NaHCO_3 + 5\ NaL}$$

$$(4)\ \frac{2.75\ H_2CO_3}{55\ NaHCO_3 + 5\ NaL} \qquad (5)\ \frac{H_2CO_3}{65\ NaHCO_3}$$

If the first ratio represents the normal acidity of the tissues the effect of the accumulation of five additional molecules of lactic acid will be shown in the second ratio. Five molecules of base will be lost and five molecules of carbonic acid formed. Assuming that sodium lactate has no buffer properties whatever the acidity is represented by the ratio 8/55. Compare this with 3/60 and assume that this change has occurred within the respiratory center and the hyperpnea of anoxemia is largely explained. The purpose of the hyperpnea is to adjust the ratio to its proper value which is shown in ratio number three. A more correct representation of conditions is shown in ratio number four. That ratio can be maintained only by continued hyperpnea. Though the carbonic acid and the $NaHCO_3$ are reduced, the NaL remains; as a matter of fact it is the important element in the maintenance of the hyperpnea, for it results in a reduction of the denominator of the acid-base ratio which increases the effectiveness of the constantly forming carbon dioxide. On admitting oxygen at ratio three, ratio five results. Only one lactate ion is oxidized; this forms three molecules of carbonic acid, giving a total of six molecules. Five of these combine with the five sodium ions that have been liberated. Such a reaction should theoretically lead to increased alkalinity; apnea should occur, and, as is well known, this is the result of administering oxygen after oxygen want.

Lack of oxygen, therefore, has a double effect on the carbonate buffer system of the muscle cell. It increases the numerator and decreases the denominator with a consequent increase in acidity, while a subsequent abundance of oxygen increases the denominator and decreases the numerator with a consequent increased alkalinity.

The questions which arise in connection with these facts are whether lactic acid can be looked upon as a normal metabolite, whether it is always present in tissues but in varying amounts depending upon the supply of oxygen and whether a similar formation and removal of lactic acid occurs in the acid metabolism of the center.

Our contribution to the subject of lactic acid metabolism was carrying the analogy of the findings on muscle to tissues in general; giving evidence for the significance of lactic acid metabolism for general tissue function (Gesell, 1923b, 1925); demonstrating the rapid formation of lactic acid in brain tissue under anaerboic conditions (McGinty and Gesell, 1925);

TABLE 1

EXPERIMENT NUMBER	GAS SAMPLE NUMBER	EXPIRED O_2	RETAINED O_2	EXPIRED CO_2	EXPIRATORY QUOTIENT	REMARKS
		per cent	*per cent*	*per cent*		
3A	1	15.29	5.65	4.89	0.87	
						12 cc. NaCN
	2	16.97	3.97	4.21	1.06	
	3	17.47	3.47	4.01	1.16	
	4	15.56	5.38	5.02	0.93	
	5	15.54	5.40	4.77	0.88	
						16 cc. NaCN
	6	17.22	3.72	4.39	1.18	
	7	16.25	4.69	5.01	1.07	
	8	15.66	5.28	4.81	0.91	
3B	1	18.32	2.62	2.57	0.98	
						11 cc. NaCN
	2	17.93	3.01	3.10	1.03	
	3	18.11	2.83	3.20	1.13	
	4	18.34	2.60	2.99	1.15	
	5	18.31	2.63	2.61	0.99	
	6	18.20	2.74	2.46	0.90	
						23 cc. NaCN
	7	19.93	1.01	2.04	2.02	
	8	20.03	0.91	1.45	1.59	
	9	19.78	1.16	1.80	1.55	
	10	19.66	1.28	1.59	1.24	
	11	18.40	2.54	2.55	1.00	
	12	17.86	3.08	3.14	1.02	
	13	17.65	3.29	3.22	0.98	
	14	17.74	3.20	2.91	0.91	
3C	1	17.16	3.78	3.51	0.93	
						21 cc. NaCN
	2	17.98	2.96	3.07	1.04	
	3	19.18	1.76	2.44	1.39	
	4	18.77	2.17	2.82	1.30	
	5	18.24	2.70	3.37	1.25	
	6	17.72	3.22	3.44	1.07	
	7	16.65	4.29	3.91	0.91	
	8	16.26	4.68	4.20	0.90	
	9	16.58	4.36	3.84	0.88	
	10	16.84	4.10	3.48	0.85	
	11	16.95	3.99	3.28	0.82	
4A	1	19.25	1.69	1.51	0.89	
						7 cc. NaCN
	2	19.74	1.20	1.12	0.93	
	3	19.02	1.92	1.68	0.87	
	4	18.95	1.99	1.97	0.99	

TABLE 3—*Concluded*

EXPERIMENT NUMBER	GAS SAMPLE NUMBER	EXPIRED O_2	RETAINED O_2	EXPIRED CO_2	EXPIRATORY QUOTIENT	REMARKS
		per cent	*per cent*	*per cent*		
4A	5	19.13	1.81	1.81	1.00	
	6	19.35	1.59	1.41	0.89	
	7	19.06	1.88	1.50	0.80	
4B	1	17.78	3.16	2.70	0.85	
						6 cc. NaCN
	2	17.61	3.33	2.90	0.87	
	3	17.88	3.06	2.87	0.94	
	4	17.86	3.08	2.68	0.87	
						19 cc. NaCN
	5	19.74	1.20	1.87	1.56	
	6	19.32	1.62	2.16	1.33	
	7	17.80	3.14	3.37	1.07	
	8	17.68	3.26	3.10	0.95	
4C	1	18.84	2.10	1.64	0.78	
						9 cc. NaCN
	2	18.84	2.10	2.05	0.97	
	3	19.15	1.79	1.85	1.03	
	4	19.25	1.69	1.51	0.89	

postulating the influence of changing balance of anaerobic and aerobic acid metabolism on the acidity of tissues as a whole, and now indicating under basal metabolic conditions a retention of carbon dioxide by the removal of lactic acid preformed in superabundance during a period of relatively anaerobic conditions. These results obtained by impaired basal metabolism followed by recovery agree with the findings on man during and following active superbasal metabolism (muscular exercise), (Long and Lupton, 1923; Hill, Long and Lupton, 1924).

The results of the second injection in figure 3B are very similar to those in figure 3C. The expiratory quotient increases from 0.98 to 2.02 during the initial impairment of oxidations and then progressively diminishes, and at sample 13 the expiratory quotient is back to 0.98 though the expired carbon dioxide is steadily increasing. The record supplies further evidence for the retention of carbon dioxide by base liberated in the lactic acid shift to the precursor state.

In the first injection of record 4A similar results occur again. The increase in the expiratory quotient is not as large, 0.89 to 1.00, but the initial decrease occurring during increasing expired carbon dioxide and the final low value of 0.80 as compared with the pre-injection value obtained. Here, too, carbon dioxide has been retained, for at the very end of the record

retained oxygen is higher (1.88 per cent) than just before injection (1.69 per cent).

In figure 4B (second injection) after the initial increase in expiratory quotient from 0.87 to 1.56 the quotient drops again though carbon dioxide elimination increases. The experiment was cut too short to know whether or not the expiratory quotient would have fallen to a lower value than 0.87, the pre-injection value.

In figure 4C overshooting of retained oxygen never occurred. Consequently according to theory there was no mechanism for the retention of carbon dioxide. This may account for the relatively high expiratory

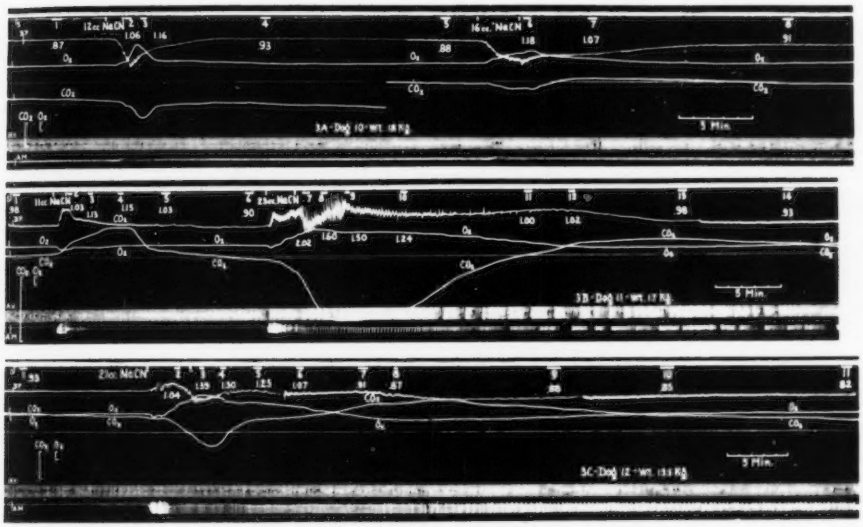

Fig. 3

quotient at the end of the record (0.89 as compared with the pre-injection level of 0.78). It should be recalled that this curve corresponds with type II response in which the secondary carbon dioxide overshooting occurs in the absence of overshooting of retained oxygen. The overshooting of carbon dioxide cannot be explained by excess oxidations for these are wanting. The increased carbon dioxide elimination may, therefore, be due to a preceding period of impaired oxidations giving rise to increased lactic acid which displaces carbon dioxide in the tissues, tissue fluids and blood. Other examples though not as striking are seen in both injections of figure 3A. Though the oxygen retained after both injections is never as great as before the respective injections expired carbon dioxide increased (cor-

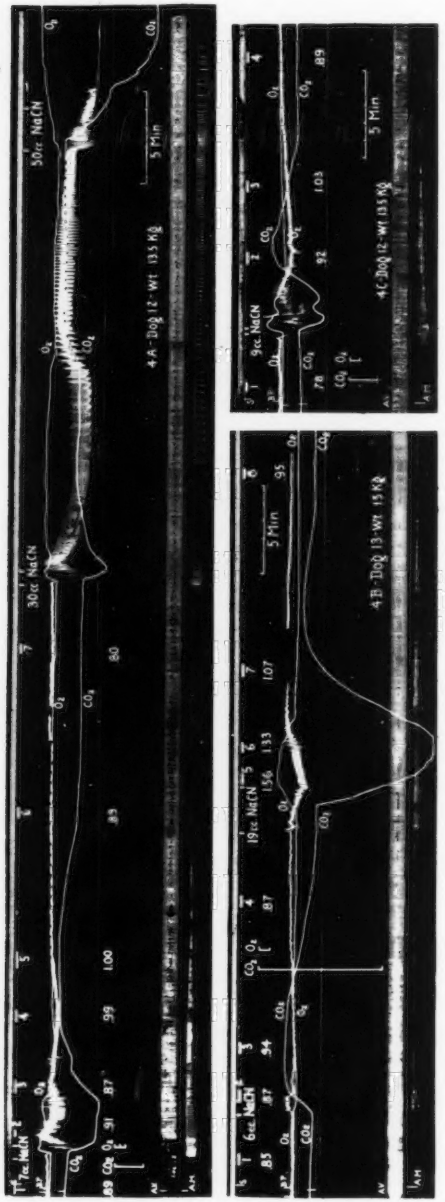
7cc. NaCN
30cc. NaCN
5 Min
4A-Dog 12-Wt 13.5 Kg.
6cc. NaCN
19cc. NaCN
5 Min
4B-Dog 13-Wt 15 Kg.
9cc. NaCN
5 Min
4C-Dog 12-Wt 13.5 Kg.

FIG. 4

rection should be made for the drift of the carbon dioxide electrode). After the first injection carbon dioxide increased to 5.02 per cent as compared with the pre-injection level of 4.89 per cent, and after the second injection it increased from 4.77 per cent to 5.01 per cent.

Obviously there are other possible explanations of the carbon dioxide overshooting. It may be due to a simple increased elimination of carbon dioxide resulting from increased volume-flow of blood which is known to occur on the administration of sodium cyanide (Bernthal, Bronk, Cordero and Gesell, to be published). Likewise, it might be due to a disturbance in the mechanism of the lactic acid shift in a direction leading to greater oxidation of lactic acid and less direct transfer to the precursor state. The ratio of lactic acid oxidized to lactic acid removed has been found to differ in individual animals. We, therefore, suggest that the ratio may change with changing conditions in the same animal. This point we hope to follow by quantitative analyses in future experiments.

We have yet to consider type IV response to administration of sodium cyanide (figs. 2B, 2A, 2C, 1F, 1G, and the first injection of 4B and 3B). These are unexpected results which require further study. It will be noticed first of all that this type of response was always obtained at the beginning of an experiment and with a relatively small injection. The injections were associated primarily with an increase in blood pressure and also with increased respiratory movements. Figures 2B, 2C, 3B and 4B all show prompt recovery of respiratory movements to pre-injection activity. In two experiments in which gasometric analyses were made (figs. 3B and 4B) the expiratory quotient was found increased. The injections may be of a magnitude which will centrally excite respiratory movements with only a minor effect on the oxidative mechanism within the muscles themselves. If that be true, the increase in oxygen retention might be accounted for by increased activity of the muscles and of the heart provided volume-flow is accelerated as it is with larger injections. The increase in expiratory quotient if not of metabolic origin may be a result of increased volume-flow of blood leading to better ventilation. Until more significant data are accumulated further speculation is unwarranted.

Discussion. In a paper which is to follow shortly on the effects of intravenous injection of sodium cyanide on blood acidity (Gesell and Hertzman), evidence was presented contradictory to the inverse relation between pulmonary ventilation and rate of oxidations. Though ventilation was stimulated as in these experiments during the initial period of decreased oxidations they were secondarily increased during the period of recovery of oxidations. This same phenomenon is apparent in our expired gas experiments and supports the postulate that oxidation is essential to rhythmic discharge of nerve cells.

In the paper on blood acidity the lack of correspondence between pul-

monary ventilation and changes in blood acidity was also pointed out. It was shown, for example, that cyanide when administered during constant artificial ventilation may produce either no immediate change in blood acidity or an increased acidity and when administered with the chest intact during normally controlled ventilation it was shown that it produces a sharp increased alkalinity. The immediate effect on respiratory movements, however, is the same. It was, therefore, proposed that the acidity of the interior of the respiratory neurones was more significant than the other factors considered, and the results were analyzed with respect to the possibility of increased acidity of the tissues from cyanide administration. It was pointed out that cyanide theoretically should lead to a change in acid metabolism with anaerobic acid formation increasing with respect to aerobic acid metabolism. This linked with a broken coördination of the dual function of hemoglobin should increase the acidity of the cells. In our expired gas experiments supporting evidence of the significance of the ratio of anaerobic to aerobic acid metabolism is presented. The results appear to agree with an acid mechanism of the control of respiration. It is, of course, difficult to predict with certainty on the basis of indirect evidence such as blood acidity and gaseous exchange the acidity changes occurring in the cell, yet the data obtained in these as in other experiments seem to conform with an acid mechanism of control. It is suggested in this connection that the results of the expired gas experiments and of the blood acidity experiments showing effects of sodium cyanide be compared.

SUMMARY AND CONCLUSIONS

The effects of intravenous administration of M/100 sodium cyanide on expired carbon dioxide and expired oxygen during constant artificial ventilation were followed with continuous electrometric methods and gasometric analyses.

Four types of response were obtained. In type I sodium cyanide elicited first a decrease in expired carbon dioxide and retained oxygen which gave way with oxidative recovery to secondary overshooting of expired carbon dioxide and retained oxygen. This was followed by an approximate return to pre-injection conditions.

In type II the response was very similar. The essential difference was the absence of the secondary overshooting of retained oxygen though overshooting of expired carbon dioxide still occurred.

In type III the secondary overshooting of both expired carbon dioxide and retained oxygen were missing.

In type IV response which was elicited by relatively small injections with the animal in good condition there was an immediate or primary overshooting in expired carbon dioxide and retained oxygen.

Type I response is similar to the usual changes produced by hemorrhage and reinjection and by administration of low oxygen and readministration of room air. The results were accounted for by a reversibility of tissue metabolism. It was suggested that the initial impaired oxidations leading to accumulation of anaerobic metabolites are followed by a compensatory period of increased aerobic metabolism, which removes the excess anaerobic metabolites.

Type III response in which secondary overshooting of expired carbon dioxide and retained oxygen are missing resemble the responses to administration of low oxygen and subsequent administration of room air in which reversibility of acid metabolism failed to develop after the period of impaired metabolism. The cyanide results of type III response were explained by permanent impairment of the oxidative mechanism of the cell.

Type II response was considered as intermediate between type I and III.

Only very tentative explanations of type IV response were offered.

During the initial decrease in expiratory exchange in types I, II and III the expiratory quotient increased. During the recovery phase it diminished. In some experiments it fell considerably below the pre-administration level in others it remained above that level. The decrease in expiratory quotient may occur while carbon dioxide elimination is considerably above normal.

The significance of these changes in the expiratory quotient was discussed in relation to the balance of anaerobic and aerobic acid metabolism. The increased expiratory quotient during the initial decreased expiratory exchange is believed to agree with an increased anaerobic production of fixed acid. The subsequent decreased expiratory quotient is believed to agree with retention of carbon dioxide and with the dual mechanism of oxidative and non-oxidative removal of lactic acid.

With a vigorous and highly reversible response to sodium cyanide the lowering of the expiratory quotient during a period of excess carbon dioxide elimination indicated relative retention of carbon dioxide and further reduction of the quotient during the subsequent period of lowered carbon dioxide elimination indicated absolute retention.

The secondary overshooting of carbon dioxide in type II response in which secondary overshooting of retained oxygen is missing was explained by a relative increase in the ratio of oxidative to non-oxidative removal of lactic acid; to the displacement of carbonic acid by the accumulation of anaerobic acids; and to the increased volume-flow of blood which may lead to greater elimination of carbon dioxide independent of increased retention of oxygen.

The results on expired gases support the explanation of changes in acidity of the circulating blood produced by cyanide (unpublished).

They appear to agree with an acid mechanism of respiratory control.

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RELATION OF THE pH OF THE BLOOD TO THE IRRITABILITY OF THE VASOCONSTRICTOR MECHANISM

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From the Department of Physiology, the University of Chicago

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In a recent paper it was shown that the response of the vascular system of the pithed cat to adrenalin varies directly with the pH of the blood (Burget and Visscher 1927). The more alkaline the reaction of the blood, within the range of pH 6.85 to 8.0, the greater is the hemodynamic response to small intravenous injections of the drug. Two possibilities were suggested for this enhanced action in a more alkaline medium, namely, 1, more complete and more rapid oxidation of the drug at a high pH, 2, an altered irritability of the sympathetic myoneural junction whereby sensitivity is increased as the blood becomes more alkaline. In the present report evidence is offered to show that this difference in response is due to change in the irritability of the structures acted upon by adrenalin, rather than to any effects produced by a change in the rate of oxidation of this substance in the vascular system.

For details of preparing the animals, and for the precautions to be taken in administering artificial respiration, as well as for references to the literature, the reader will be asked to consult the earlier paper.

Blood pressure was registered from a carotid artery, while on the opposite side the cervical sympathetic cord was isolated. A cannula was placed in one femoral artery for collection of blood samples and the femoral vein on the other side was cannulized and connected by rubber tubing with a burette containing 0.9 per cent NaCl solution. Adrenalin (Parke, Davis & Co.) was diluted to 1–100,000 and injected in 0.2 cc. doses into the rubber tubing, whence it was carried into the circulatory system by letting 2 cc. of normal saline flow into the vein from the burette. The dose was accurately controlled by using a graduated 1 cc. pipette, on one end of which was a hypodermic needle and on the other a 5 cc. syringe.

In order to investigate whether or not any changes in the sensitivity of the sympathetic myoneural junction took place with alterations in the pH of the blood, the sympathetic cord in the neck was stimulated with a medium faradic current and the vasoconstrictor effect on general blood pressure registered. The strength of the stimulating current was kept constant so that any variation in response with variation in pH of the

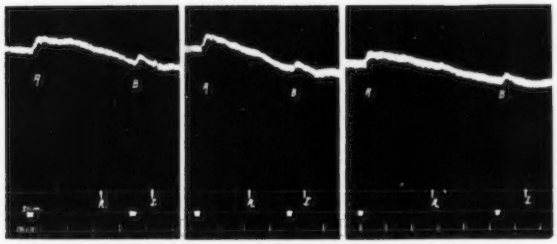
blood would indicate a change in irritability of the tissues involved. The pH of the blood was caused to vary by altering the ventilation of the animal. If the ventilation is slightly inadequate there is a gradual accumulation of CO_2 in the blood and a fall in pH, while ventilation increased beyond normal washes out the CO_2 and causes the pH to rise.

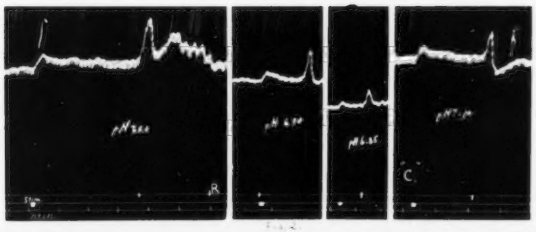
Following the adrenalin injection and stimulation of the cervical sympathetic with a medium faradic current, a pH determination of the plasma was made, observing the following technic: a 1.5 to 2.0 cc. sample of blood was drawn under paraffin oil into a centrifuge tube in which one drop of a saturated potassium oxalate solution had been evaporated. The oxalate was distributed through the sample by gently rolling the tube, after which it was placed in the centrifuge to bring down the cells. A pH determination was then made on the plasma by the colorimetric method at body temperature as described by Hastings and Sendroy (1924).

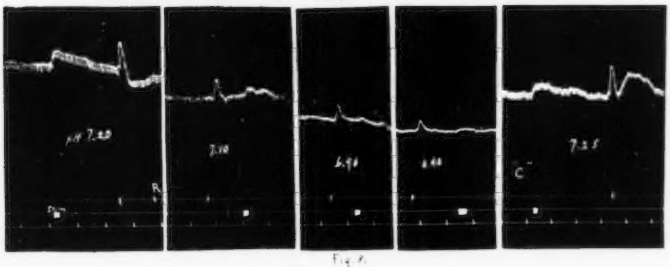
Figure 1 shows the effect upon general blood pressure of stimulation of the cervical sympathetic under conditions of *1*, ample ventilation, and *2*, following two or three minutes of reduced ventilation. The difference in response is distinct and indicates that under ample ventilation faradic stimulation is more effective. In figures 2 and 3 both the response to faradic stimulation of the cervical sympathetic and the response to adrenalin injection are shown and the pH of the blood at the given time indicated. The adrenalin reactions conform to those reported earlier, namely, as the pH falls the response decreases; the effect of cervical sympathetic stimulation with a faradic current varies in a similar manner, that is, the effectiveness of the latter increases as the pH of the blood rises and decreases as the pH falls.

Andrus (1924) working with the isolated auricle of the rabbit, found that the action of adrenalin was enhanced when the surrounding fluid was more alkaline than normal and reduced when it was less alkaline. In order to test whether or not this difference in reaction was due to a change in susceptibility of the adrenalin to oxidation or to alteration of susceptibility of the tissue, he used the more stable tyramine hydrochloride in place of adrenalin. This drug was also found to be more effective in the more alkaline medium. He concludes that "in as much as tyramine hydrochloride is not spontaneously oxidizable it is probable that the effect of changes in pH upon its action is due to a change in the responsiveness of the tissues."

Thus Andrus' experiments tend to exclude the possibility of change in oxidation of adrenalin with a change in pH of the medium as being a factor in altering its effectiveness as a stimulus, and point to a change in tissue irritability as the cause. Our work furnishes direct evidence that the irritability of the structures acted upon by adrenalin is altered by variation of the pH of the blood.







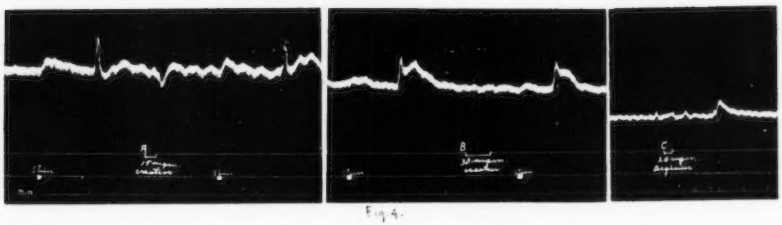


Fig. 1. Showing the diminished effect of cervical sympathetic stimulation upon blood pressure following reduced ventilation. R = reduced ventilation; I = increased ventilation; A = the effect following ample respiration; B = the effect following inadequate respiration.

Figs. 2 and 3. Tracings from two different animals each showing the effects of both cervical sympathetic stimulation and adrenalin (0.2 cc. of 1:100,000) intravenously upon blood pressure at different pH values of the blood. Ventilation reduced at R; C, after one hour of ample ventilation.

Fig. 4. The effect of creatin and arginin upon the vascular response to both cervical sympathetic stimulation and adrenalin injection (0.2 cc. of 1:100,000). A, 15 mgm. creatin injected intravenously; B, 30 mgm. creatin; C, 20 mgm. arginin.

While we were investigating this problem Brodd (1927) reported that an increased effectiveness of adrenalin in bringing about vasoconstriction of the vascular system of the frog followed the addition to the perfusing liquid of either creatin or arginin. We have found no alteration of the adrenalin response following intravenous injection of either of these substances. Figure 4 illustrates the ineffectiveness of both creatin and arginin in altering the adrenalin reaction of the vascular system of the pithed cat.

CONCLUSION

1. Variations in the response of the vascular system to adrenalin with changes in the pH of the blood are due to alterations in the irritability of the myoneural junction rather than any change of effectiveness brought about by variation of the susceptibility of the drug to oxidation.

2. Intravenous injection of creatin or arginin does not alter the response of the vascular system of the pithed cat to injections of adrenalin.

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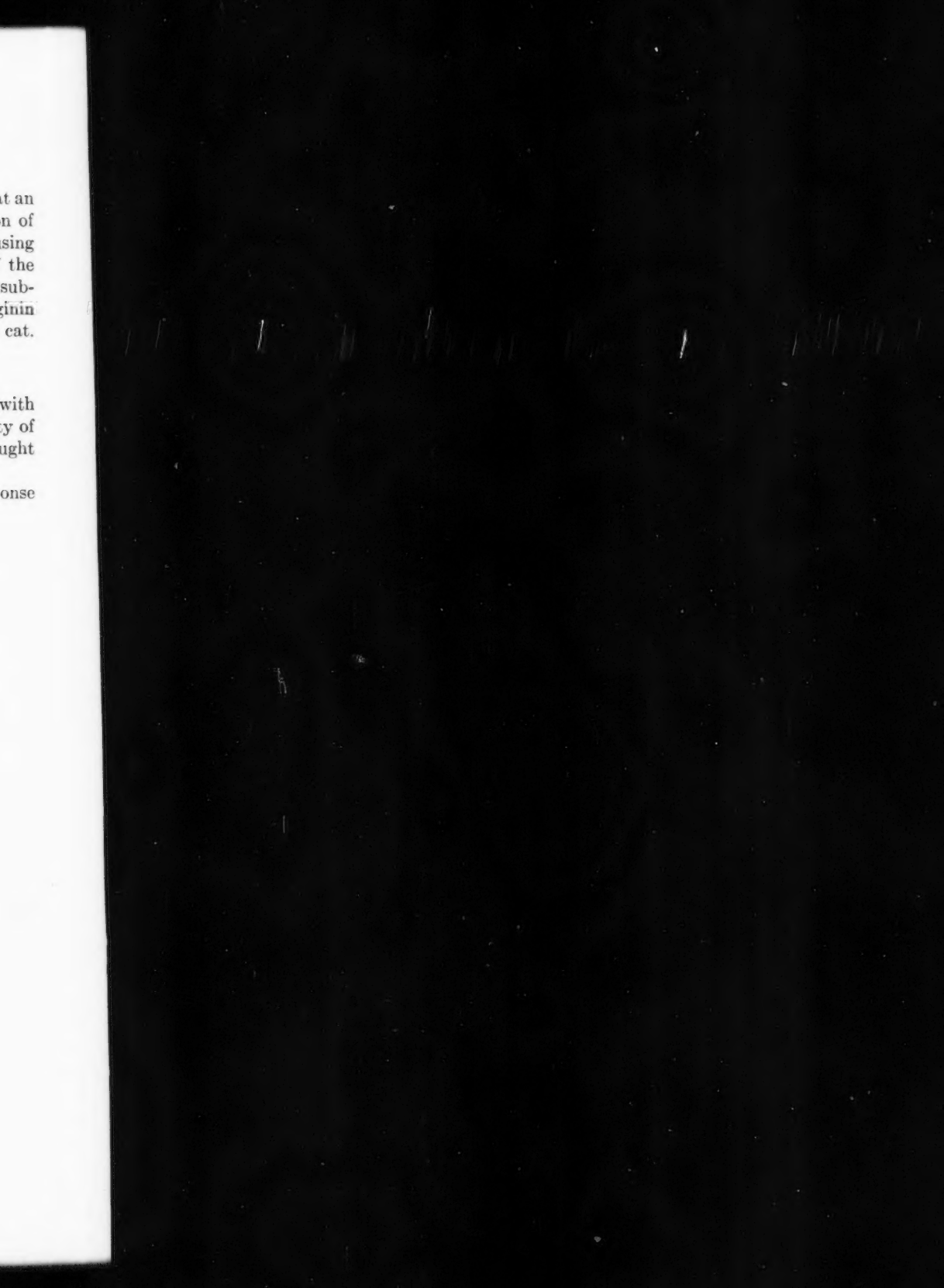

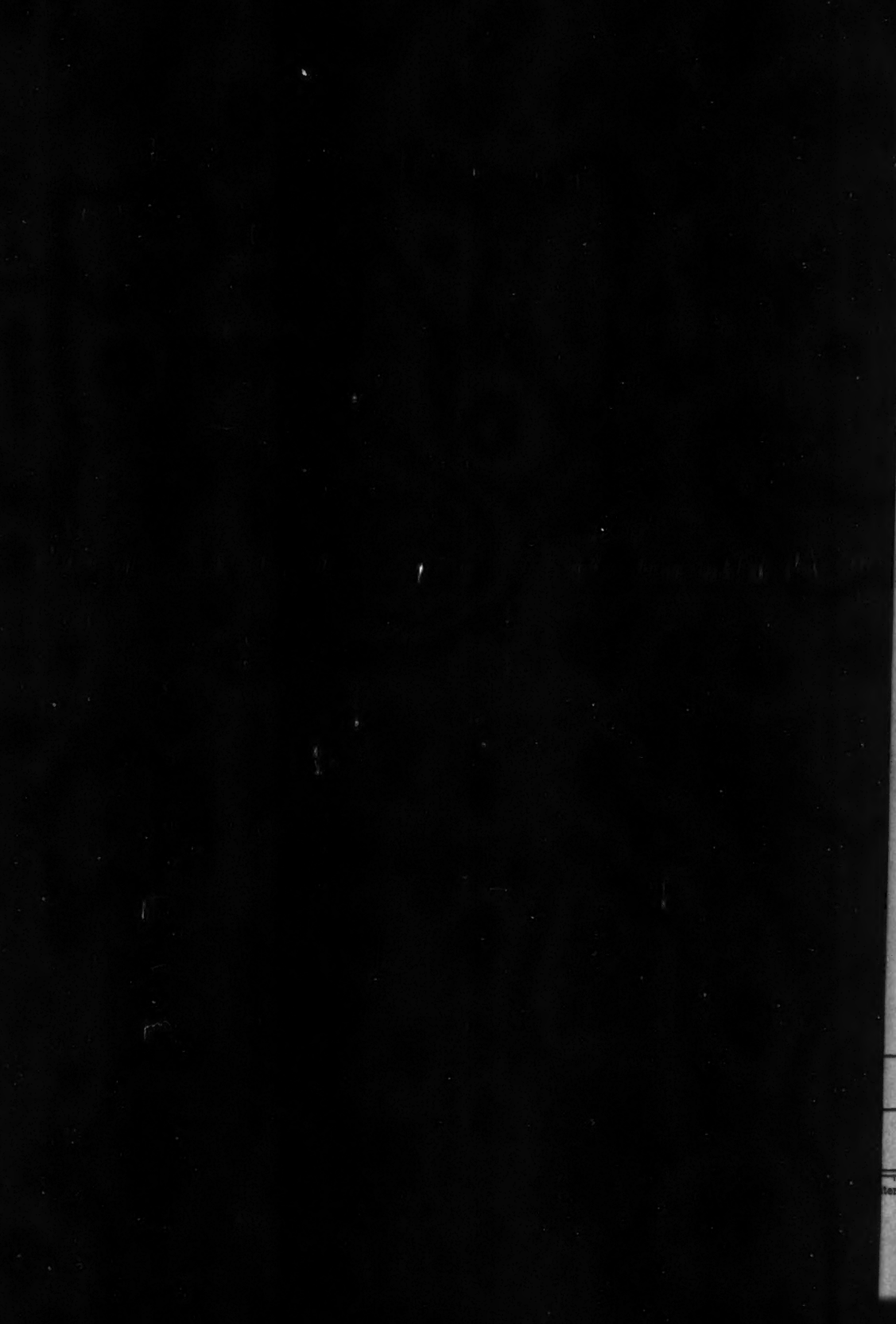

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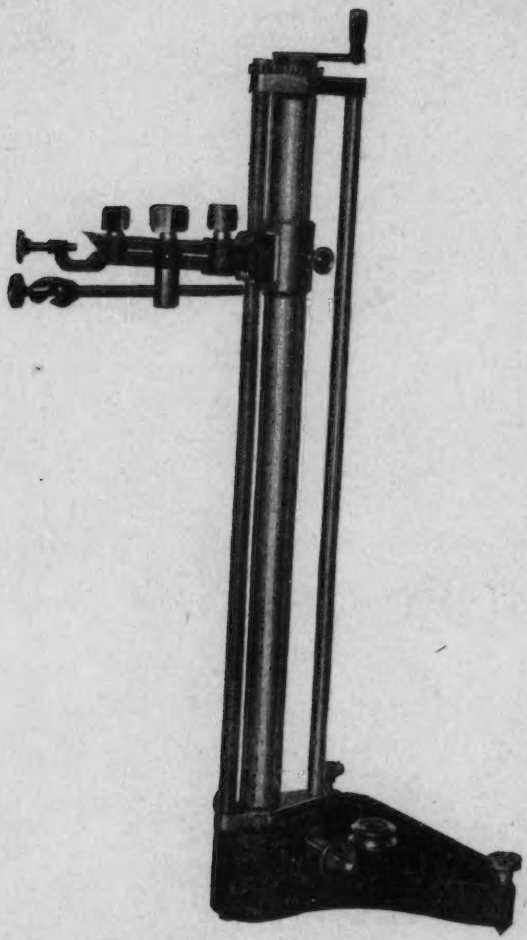

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